CR 92.008

# NCEL

Contract Report

JUNE 1992

An Investigation Conducted by
Dr. Eve Riser-Roberts

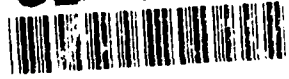
# IN SITU/ON-SITE BIODEGRADATION OF REFINED OILS AND FUELS (A Technology Review)

## VOLUME 3



## APPENDICES B TO F





NAVAL CIVIL ENGINEERING LABORATORY PORT HUENEME CALIFORNIA 93043-5003

## METRIC CONVERSION FACTORS

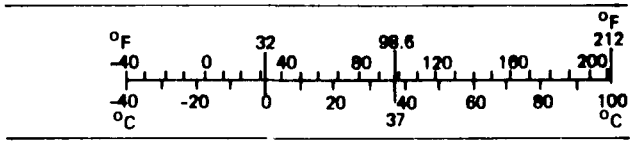
### Approximate Conversions to Metric Measures

| Symbol | When You Know | Multiply by | To Find | Symbol |
|---|---|---|---|---|
| | | **LENGTH** | | |
| in | inches | *2.5 | centimeters | cm |
| ft | feet | 30 | centimeters | cm |
| yd | yards | 0.9 | meters | m |
| mi | miles | 1.6 | kilometers | km |
| | | **AREA** | | |
| $in^2$ | square inches | 6.5 | square centimeters | $cm^2$ |
| $ft^2$ | square feet | 0.09 | square meters | $m^2$ |
| $yd^2$ | square yards | 0.8 | square meters | $m^2$ |
| $mi^2$ | square miles | 2.6 | square kilometers | $km^2$ |
| | acres | 0.4 | hectares | ha |
| | | **MASS (weight)** | | |
| oz | ounces | 28 | grams | g |
| lb | pounds | 0.45 | kilograms | kg |
| | short tons (2,000 lb) | 0.9 | tonnes | t |
| | | **VOLUME** | | |
| tsp | teaspoons | 5 | milliliters | ml |
| Tbsp | tablespoons | 15 | milliliters | ml |
| fl oz | fluid ounces | 30 | milliliters | ml |
| c | cups | 0.24 | liters | l |
| pt | pints | 0.47 | liters | l |
| qt | quarts | 0.95 | liters | l |
| gal | gallons | 3.8 | liters | l |
| $ft^3$ | cubic feet | 0.03 | cubic meters | $m^3$ |
| $yd^3$ | cubic yards | 0.76 | cubic meters | $m^3$ |
| | | **TEMPERATURE (exact)** | | |
| °F | Fahrenheit temperature | 5/9 (after subtracting 32) | Celsius temperature | °C |

*1 in = 2.54 (exactly). For other exact conversions and more detailed tables, see NBS Misc. Publ. 286, Units of Weights and Measures, Price $2.25, SD Catalog No. C13.10:286.


### Approximate Conversions from Metric Measures

| Symbol | When You Know | Multiply by | To Find | Symbol |
|---|---|---|---|---|
| | | **LENGTH** | | |
| mm | millimeters | 0.04 | inches | in |
| cm | centimeters | 0.4 | inches | in |
| m | meters | 3.3 | feet | ft |
| m | meters | 1.1 | yards | yd |
| km | kilometers | 0.6 | miles | mi |
| | | **AREA** | | |
| $cm^2$ | square centimeters | 0.16 | square inches | $in^2$ |
| $m^2$ | square meters | 1.2 | square yards | $yd^2$ |
| $km^2$ | square kilometers | 0.4 | square miles | $mi^2$ |
| ha | hectares (10,000 $m^2$) | 2.5 | acres | |
| | | **MASS (weight)** | | |
| g | grams | 0.035 | ounces | oz |
| kg | kilograms | 2.2 | pounds | lb |
| t | tonnes (1,000 kg) | 1.1 | short tons | |
| | | **VOLUME** | | |
| ml | milliliters | 0.03 | fluid ounces | fl oz |
| l | liters | 2.1 | pints | pt |
| l | liters | 1.06 | quarts | qt |
| l | liters | 0.26 | gallons | gal |
| $m^3$ | cubic meters | 35 | cubic feet | $ft^3$ |
| $m^3$ | cubic meters | 1.3 | cubic yards | $yd^3$ |
| | | **TEMPERATURE (exact)** | | |
| °C | Celsius temperature | 9/5 (then add 32) | Fahrenheit temperature | °F |



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**13. ABSTRACT** *(Maximum 200 words)*

This extensive literature review covers all aspects of in situ and on-site bioremediation of gasoline, middle distillate fuels, and other refined petroleum products, using information available through 1988. Topics covered include: composition of different fuels, factors affecting biodegradation rates, responsible microorganisms, degradation pathways, factors enhancing biodegradation, and potential limitations. This report is in three volumes. Volume 1 covers general information of most interest to managers and non-specialists. Volume 2 contains technical information required for in-depth coverage. Volume 3 covers related information, including detailed applied information on screening and microbial enumberation methods, laboratory microorganisms studies, bioreclamation site preparation, bioreactor use and selection, cost data, and competing technologies.

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## DISCLAIMER

The following is a sample of the types of programs being used for bioremediation. This is only a partial list of companies involved in this work and a summary of some of the techniques they employ. The information is presented to introduce the reader to the variety of processes and materials being used commercially for this application. The information was derived from papers available to the author at the time the report was written in 1988. Mention of these companies does not constitute or imply an endorsement, recommendation, or approval of any of these companies or their programs or products.

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# SECTION 1

# COMMERCIAL PROGRAMS

## 1.1 FMC AQUIFER REMEDIATION SYSTEMS

The Aquifer Remediation Systems (ARS) Bio XL process employs stabilized solutions of hydrogen peroxide (tradename Restore) to increase the amount of oxygen in the soil by more than 25 times, in comparison with air sparging, an earlier method. Another of its products (Restore Microbial Nutrient) prevents precipitation of chemical nutrients. Bioreclamation can now be used in low-permeability formations, where the pumping rate from recovery wells is as low as 5 gal/min. Table B-1 shows the costs associated with this company's process.

The company's remediation technique, Enhanced Bioreclamation, takes advantage of natural soil bacteria to degrade many organic compounds to carbon dioxide and water (Brubaker and O'Neill, 1982). The process involves introduction of microbial nutrients into the contaminated area to convert it into a bioactive zone of biodegradation. It is not the complete answer for every combination of hydrogeologic conditions or chemical contaminants, but it promises to become a powerful technique in remediating certain types of sites. The technique requires an understanding of the hydrogeology, chemistry, and microbiology of the site. The contamination must be defined both horizontally and vertically and a pumping strategy designed for rapid and controlled movement of groundwater through the area. This may often include a reinjection system to optimize nutrient utilization and total water movement. Since biodegradation occurs in the capillary zone, the effort required in adjusting water levels is reduced.

It must be determined which materials in the site are biodegradable and what type of nutrient formulation is best suited to stimulate the bacteria and yet be compatible with the site chemistry. The project also relies on understanding the soil chemistry and the potential for interaction between the nutrients and the soil. The time and cost of the effort is affected by the amount of nutrients needed and by the rate at which the nutrient solution can be introduced into the formation. The total reservoir of contamination in the soil is an important factor in predicting time and costs. However, it can be quite expensive to obtain this information, and it is only an indication of how long the remediation process will be required. Thus, analysis of the total soil and water contamination and an estimate of sustainable pumping rates through the site become important in predicting project cost.

Enhanced Bioreclamation will usually be used in combination with other remediation techniques. This could be an auxiliary surface water treatment, such as air stripping, carbon treatment, or surface biological treatment. Carbon adsorption is most often used with aromatics (including chlorinated aromatics, phenols, and PAHs), fuels, chlorinated solvents, and high molecular weight amines, ketones, and surfactants. Larger or smaller compounds will not fit into the micropores on the surface of the carbon. A mixture of materials may not respond like the sum of the individual components; some may inhibit

Table B-1. Costs of Bioreclamation

| | Carbon adsorbers | Bioreclamation |
|---|---|---|
| Circulation rate*, $m^3/s$ | $6.308 \times 10^{-3}$ | $6.308 \times 10^{-3}$ |
| Cleanup time, yr | 10 to 20 | 0.33 to 0.66 |
| Fixed costs, $ | | |
| Project evaluation | -- | 90,000 to 70,000 |
| Construction/startup | 120,000 to 450,000 | 50,000 to 75,000 |
| Variable costs, $/yr | | |
| Chemicals | 10,000 | 90,000 to 112,000 |
| Labor | 25,000 | 6,000 to 10,000 |
| Total project cost, $ | 470,000 to 850,000 | 180,000 to 270,000 |

*Influent conc., 20 to 40 ppm

adsorption of others. Another problem is that those compounds that adsorb well to carbon also bind to the soil and may be difficult to release into the water. Surface biotreatment depends upon both the biodegradability of the material and the availability of either an existing treatment facility or the room to install a treatment facility on site. The amount of degradation that will occur above ground versus in the ground depends upon solubility of the material. If it is soluble, and there already is a biological treatment facility, it may be cheaper to treat in the extracted groundwater existing facility. Often, however, it takes much longer to first extract the material than to use the in situ technique. Table A.2-13 presents some of the common organic contaminants, their solubility in water, and their biodegradability (Brubaker and O'Neill, 1982).

FMC Aquifer Remediation Systems (ARS) has developed an adjunct to their in situ Bio XL$_{SM}$ Enhanced Bioreclamation Process for the on-site treatment of contaminated soils (FMC Aquifer Remediaiton Systems, 1986). This process, Enhanced Surface Bioreclamation (ESB), combines ARS's technology with experience in the heap-leach mining industry to provide a new, on-site treatment strategy.

The ESB process is based upon stimulation of natural degradation, controlled and uniform application, and containment of the reaction. Application of nutrients and oxygen is a critical component of the process. Nutrient concentrations and flow rates are calculated on carbon loading, treatment volume, type of contaminant, and microbial populations for optimum removal of the contaminant. Uniformity and control of nutrient application are also necessary. This requires that the soil be homogenous, permeable, and stable. To stabilize the soil matrix, the ESB process uses agglomeration technology adapted from the heap-leach mining industry. This mixes a small amount of a pozzolanic material with the contaminated soil. With very high organic loading or clay content, an additional inert support material may be added. Containment is achieved by building the agglomerated soil into a trapezoidal pile on a diked pad. The pile geometry minimizes the space needed for treatment, while the pad traps all fluids for recycle. The pad can be a permanent asphalt or concrete base, or a single-use clay base with an impervious liner. Both have a leachate monitoring system installed underneath to assure containment integrity.

The extent of a contamination incident is determined by evaluating the zones of contamination (Figure B-1) (Brown, Norris, and Brubaker, 1985). Zone 1 is an adsorbed area, created from the movement of free product through soil and the flux of the water table. Gasoline concentrations range from 500 to 10,000 ppm. It is a continuous source of groundwater contamination. Zone 2 is the area where free product floats on top of the water table. Its extent is determined primarily by the volume of the material lost and the groundwater gradient. Its removal reduces the increase in the adsorbed area. Zone 3 is the dissolved plume. It ranges in concentration up to 200 ppm for gasoline. Its extent is determined primarily by groundwater flow. It is the focus of regulatory attention.

FMC believes that hydrogen peroxide is the most efficient way to move oxygen through a formation, while other companies prefer to use aeration in their bioremediation programs (Ri[illegible], Bluestone, and Cannon, 1986). While some companies are wary of the disinfectant action of hydrogen peroxide, FMC

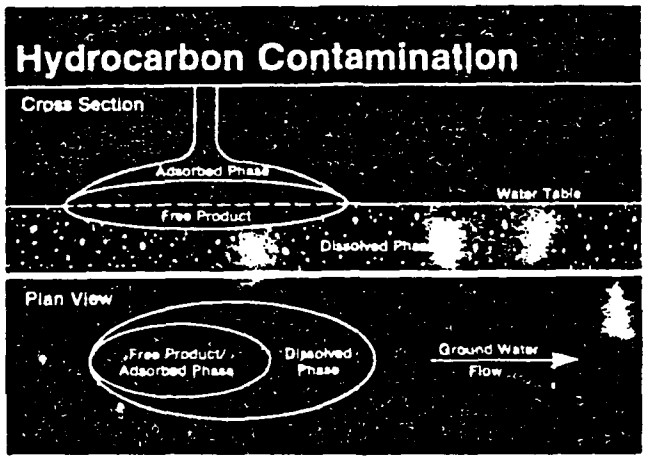


Figure B-1. Comparison of Zones of Contamination (Brown, Norris, and Brubaker, 1985)

contends that naturally occurring soil bacteria contain an enzyme to deal with the peroxide.

## 1.2 GROUNDWATER TECHNOLOGY

Groundwater Technology has a similar *in situ* process, called END (Enhanced Natural Degradation). It is planning to introduce a new system that could cut the amount of hydrogen peroxide consumption by 75 to 90 percent by modifying the oxygen delivery system into a closed loop.

Groundwater Technology first determines the feasibility of using END on each site by establishing the extent of contamination, groundwater conditions, and the quantity of hydrocarbon-utilizing microbes on site.

A cone of depression is created in the water table, which forces contaminated groundwater to flow towards the area of maximum cleanup, where it comes into contact with hydrocarbon-utilizing microbes. This system can safely clean up subsurface contamination and restore an aquifer to an acceptable quality.

To optimize growth of the hydrocarbon-utilizing microbes, laboratory tests determine the required nutrients. The microbial growth and reduction of contaminants is monitored. Treatment with END can be 80 percent that of excavation and disposal of contaminated soil, with no toxic residual by-products. After treatment, the microorganisms return to a natural biologic balance. No foreign organisms are introduced.

The program is divided into three phases:

Phase 1

The extent of contamination is determined, a monitoring system to track movement is designed, groundwater samples are analyzed, and the quantity of indigenous microbes is established. A site-specific nutrient mix of nitrogen, phosphorus, oxygen, and certain trace minerals is developed. A hydrogeologic assessment is made.

Phase 2

Using the company's technology and equipment, an END system is tailored to the specific problem. The contaminant plume is contained by installing a water table depression pump in a recovery well. Pumping groundwater from the well lowers the water table elevation, creating a cone of depression. This causes contaminated groundwater to flow towards the well, rather than off-site. Decontaminated water is then recharged back into the aquifer, carrying nutrients and oxygen to the microbes in the contaminant plume. An oil recovery systems air stripping tower removes dissolved levels of hydrocarbons in the groundwater prior to recharge. This removes over 99 percent of the dissolved hydrocarbons and adds dissolved oxygen to the plume.

Phase 3

The effectiveness of END projects is monitored through ongoing analyses of groundwater samples. This includes adjustment of nutrient variables and preparation of progress reports for regulatory agencies.

Normally, the process takes one to three years, compared with three to five years for the less effective, but more popular, pump-and-treat programs (Brooks and McGinty, 1987). The method offers the advantage of on-site cleanup and does a more complete job of cleaning water.

## 1.3 GROUNDWATER DECONTAMINATION SYSTEMS, INC. (GDS)

This company employs naturally occurring microorganisms at the site of a spill to break down biodegradable and halogenated hydrocarbons to harmless substances (Anonymous, 1984). The system works on site, and clean-up costs are $0.02/gal of wastewater.

GDS has developed a solvent refluxing system (see Appendix F, Biotechnologies in Remediation) to eliminate hydrocarbons and halogenated hydrocarbon contaminants from groundwater and soil. The company has employed this system to reclaim a site in Linden, NJ.

The GDS process involves pumping contaminated groundwater into activating tanks where the microorganisms found in the water are enriched with compounds of phosphates and ammonia. Trace amounts of inorganic salts of iron, manganese, and magnesium are also sometimes added. From the activating tanks, the water is transferred to settling tanks. Treated water, rich in oxygen, nutrients, and microorganisms is pumped into trenches for recirculation throughout the site. This permits the biodegradation process to occur in situ, as well as in the tanks. Aeration of the groundwater and soil through air injection wells further increases the rate of biodegradation. The combination of above-ground and in-ground systems permits highly detailed monitoring of the entire process (see Figure B-2) (Groundwater Decontamination Systems, Inc.). The essential novelty in the process is the extended biological surface treatment (16 hours) under optimum conditions (Rich, Bluestone, and Cannon, 19865).

It is essential that laboratory studies be made of the contaminated soil and groundwater to determine the optimum conditions for maximum biodegradation. When optimum growth conditions are maintained, the bacteria grow exponentially and increase in number as much as 10,000 times, thus greatly accelerating the rate of biodegradation. The GDS system decontaminates both soil and groundwater by accelerating nature's own process of biodegradation. The system operates on site, with a minimum disruption of normal business activity and traffic. Materials are not hauled away to distant locations. GDS tests and evaluates the types of contaminants to determine whether the system can eliminate them effectively before the project is begun. Complete cleanup costs are less than those of any other method. Complete cleanup time can be a matter of years, instead of decades with other methods. The company maintains a pilot plant and a New Jersey DEP-certified water pollution laboratory to determine the applicability of their system for a particular contaminated site.

With the GDS process, reduction of contaminant levels in the activation tanks occurs at a rate of 98 percent or better. Initially, the overall reduction of contaminants on site occurs at a greater rate above ground in the activation tanks; as the contaminant levels drop, most of the decontamination occurs in the ground. The cost is less than two cents per gallon.

The company offers the following services:

1. Licensing

   Their consultants will help set up and run a GDS system. The license fee is based upon the capacity and complexity of the system involved.

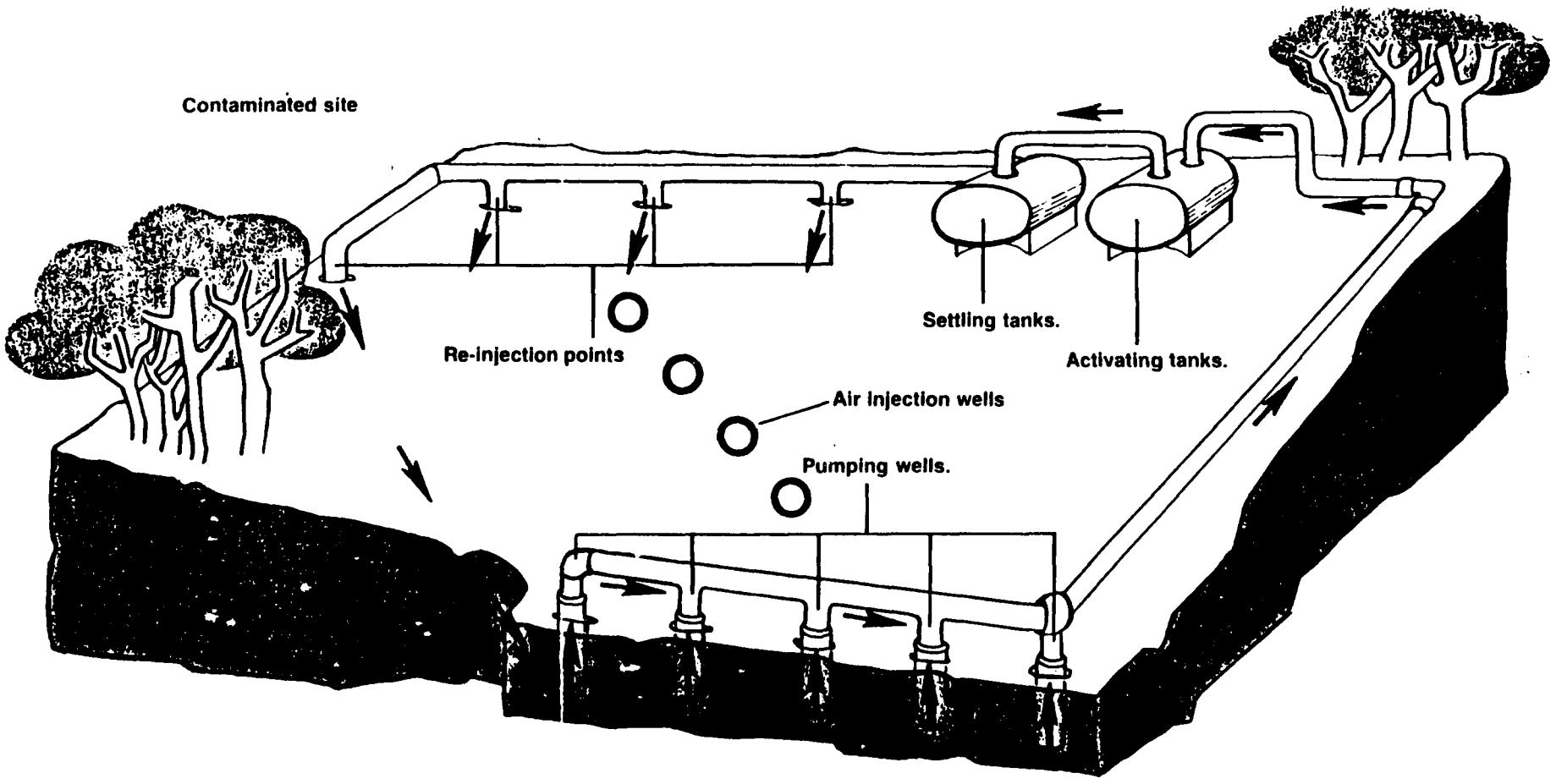


Figure B-2. GDS System (Environmental Protection Agency, 1985c)

2. Design

   They can design an entire system that will specifically meet the characteristics of the site and the contaminants present.

3. Construction

   GDS can design, engineer, and construct an entire system to solve individual contamination problems.

4. Monitoring

   GDS will monitor an installation and train the personnel to assure its efficient operation.

This company believes that air is the most cost-effective oxidizing agent and uses aeration in its bioreclamation systems (Rich, Bluestone, and Cannon, 1986).

Ground Water Decontamination Systems undertook a bioreclamation project for the Biocraft plant in New Jersey. This system will require less than the estimated five years to clean up the site, at a savings of 90 percent over alternative cleanup methods. Project and operating costs for treating an aquifer contaminated with methylene chloride, n-butyl alcohol, dimethyl aniline, and acetone are explained below (Jhaveria and Mazzacca, 1982; Jhaveri and Mazzacca, 1985).

Total costs for the biostimulation project:

| | | |
|---|---|---|
| 1. | Research and development | $453,399.00 |
| 2. | Hydrogeological design and construction | 184,243.00 |
| 3. | Process plant design and construction | 221,207.00 |
| | | $858,849.00 |

The total dollars spent include all aspects of the three individual costs. Elimination of the cost of the learning curve projects the cost at the Biocraft site to be approximately $300,000.00.

Based upon treating 13,500 gpd, the total operating cost, including utilities, nutrient cost, labor, maintenance, and overhead, was $0.0165/gal. The total cost, including amortization based on projected cost, was $0.0358/gal over a three-year period.

After three years, capital costs were $926K, and the Operating and Maintenance costs were $226/day (Environmental Protection Agency, 1984b). The total cost with biodegradation was estimated to be one-fourth that via the initial remedial measure (pumping and off-site disposal) (Amdurer, Fellman, and Abdelhamid, 1985). The preliminary research and pilot studies accounted for about $450,000, or one-half of the total capital cost. A significant investment is necessary in site-specific research. Treatability studies and research are a fundamental part of in situ remediations.

After five years, total charges for the project had come to about $40/cu yd of fully decontaminated soil (Rich, Bluestone, and Cannon, 1986). This compares favorably with other methods of soil treatment, including bioreclamation. Capital costs were $405,000, and $520,000 was spent on research and development. Costs for treating 13,600 gal of groundwater daily were 1.65 cents/gal, in contrast with 35 cents/gal that Biocraft had previously paid to have the groundwater shipped off-site for disposal. The affiliated company was Ground Water Decontamination Systems, Waldwick, NJ.

## 1.4 DORR-OLIVER

An aerobic fluidized-bed biological system has been developed to remove organics and solvents from contaminated aqueous streams (Oxitron system). The system combines the features of activated sludge and fixed-film biological processes (see Appendix F, Biotechnologies in Remediation).

## 1.5 ECOLOTROL, INC.

An aerobic fluidized-bed biological system has been developed to remove organics and solvents from contaminated aqueous streams. The system combines the features of activated sludge and fixed-film biological processes (see Appendix F, Biotechnologies in Remediation).

## 1.6 POLYBAC

Mutant bacterial formulations have been developed by Polybac Corporation to degrade the most complex organic materials, including industrial surfactants, crude and refined petroleum products, pesticides and herbicides, and solvents (Thibault and Elliott, 1979). Polybac maintains an up-to-date library of information on the relative biodegradability of a wide range of organic chemicals by various mutant strains. This allows selection of the proper formulation.

Bacterial strains are often isolated by the company from locations where natural selection has already produced a population able to grow on unusual substrates. These isolates are purified, subjected to mutagenic agents (radiation or chemicals), tested for safety, adapted to specific compounds by growth in a culture medium containing progressively higher concentrations of the particular contaminant, and freeze-dried for long-term storage. The final product is a mixture of mutant strains designed for a particular purpose and which can be reconstituted by the addition of water.

Polybac Corporation's products have been used to reduce formaldehyde levels in the ground from 1,000 ppm to under 50 ppm within 50 days, to clean up oil spilled onto surface soils and a beach (McDowell, Bourgeois, and Zitrides, 1980), to treat wastewater containing hazardous organics (Wilkinson, Kelso, and Hopkins, 1978), to restore soils and groundwater contaminated by acrylonitrile (Polybac Corp., 1983), and remove orthochlorophenol, which polluted soil and a pond (Anonymous, 1981).

Some of the company's products for bioreclamation are: PHENOBAC$^{R}$ Mutant Bacterial Hydrocarbon Degrader and PETROBAC$^{R}$ Mutant Bacterial Hydrocarbon Degrader, mutant bacterial formulations; POLYBAC$^{R}$ E Biodegradable Emulsifier, a synthetic biodegradable emulsifier; POLYBAC$^{R}$ N Biodegradable Nutrients, a proper balance of nitrogen and phosphorus.

This company is studying the use of microbes for metals removal from wet-scrubber blowdown streams.

Polybac has also marketed a biological system for treatment of phenolic wastewaters (Roberts, Koff, and Karr, 1988). This system consists of a stirred-tank bioreactor that contains PVC packing and has reportedly been used to treat wastewater from paint removal/aircraft maintenance operations.

## 1.7 O'KELLEY COMPANY

This company is studying the use of microbes for metals removal from wet-scrubber blowdown streams (Bove, Lambert, Lin, Sullivan, and Marks, 1984).

## 1.8 B.C. RESEARCH

This company has been investigating microbial copper leaching. It has been able to remove 95 percent of the copper from 600-gram batches, with recovery of elemental sulfur. The company plans to develop this into a 2- to 10-tpd pilot system. However, it is not expected to be commercially usable within the next five to ten years (reported 1983; Short and Parkinson, 1983).

## 1.9 MCGILL UNIVERSITY

McGill University has patents pending on a number of microbial formulations that recover metals from dilute aqueous streams (Bove, Lambert, Lin, Sullivan, and Marks, 1984).

## 1.10 SUNTECH

In 1972, it was recommended that the addition of nutrients to stimulate degradation of hydrocarbons in groundwater might be a feasible solution to aquifer pollution problems (Davis, et al., 1972). Suntech was probably the first to put these suggestions into practice. The company received a patent in 1974 entitled "Reclamation of Hydrocarbon Contaminated Ground Waters" on a process to eliminate hydrocarbon contaminants in aquifers by providing nutrients and oxygen to the hydrocarbon-utilizing microorganisms present in the groundwater (Raymond, 1974). The nutrients and oxygen are introduced through wells and circulated through the contaminated zone by pumping one or more producing wells. Supplementation of the nutrient and oxygen levels and recirculation of the water would allow the normal microbial flora to decompose the hydrocarbons more rapidly than they could under natural conditions. The nutrient solution would contain sources of nitrogen, phosphorus, and other inorganic salts, if necessary, at concentrations of 0.005 to 0.02 percent, by weight, for each of the nutrients. Oxygen would be supplied by sparging air into the groundwater. The process was expected to be largely complete within six months. Return to the normal levels of bacteria would occur after nutrient addition was stopped, and since no organisms were added, the normal flora would be maintained.

This process has been largely used to clean up gasoline-contaminated aquifers. The following steps are involved. Physical methods are employed to recover as much of the gasoline as possible (Suntech, Inc., 1977). If practical, pumping contaminated wells can be continued to contain the gasoline (Raymond, Jamison, and Hudson, 1976). Then investigations of the hydrogeology and the extent of contamination should be made (Suntech, Inc., 1977).

A laboratory study must then be conducted to determine if the native microbial population can degrade the components of the spill and what kinds and amounts of inorganic salts are required to stimulate degradation (Raymond, 1978). The laboratory study determines what combination of nutrients gives the maximal cell growth on gasoline in 96 hr at the ambient temperature of the groundwater. Considerable variation in the nutrient requirements has been found among aquifers. One system required only the addition of nitrogen and phosphorus sources (Raymond, Jamison, and Hudson, 1976), while the growth of microbes in another aquifer was stimulated best by the addition of ammonium sulfate, mono- and disodium phosphate, magnesium sulfate, sodium carbonate, calcium chloride, manganese sulfate, and ferrous sulfate (Raymond, Jamison, and Hudson, 1978). The form of the nutrient that must be added also varies; ammonium sulfate gave much greater growth than ammonium nitrate in one aquifer system. Chemical analyses of the groundwater provide little information as to the nutrient requirements for the system.

After the microbial investigation has established the optimal growth conditions, the system for injecting the nutrients and oxygen and producing water to circulate them in the formation must be designed and built (Raymond, Jamison, and Hudson, 1976). This work should be under the direction of a competent groundwater geologist since controlling the groundwater flow is critical to the success of the operation. Placement of the injection and production wells, such that groundwater flow goes through the contaminated zone, is required. Recycling the contaminated water from the producing wells is suggested, since it eliminates the problem of waste disposal and allows the

recirculation of unused nutrients. The screens for the wells should be large enough to permit fluctuations of the groundwater table due to weather conditions or operation of the system.

Once actual operations are underway, nutrient addition can be by batch or continuous feed. Batch addition gives satisfactory results and is more economical. For one system, the nutrients were prepared as a 30 percent concentrate in a tank truck and injected into the aquifer (Raymond, Jamison, and Hudson, 1976). This may have resulted in osmotic shock to the microorganisms that came into contact with the concentrate before dilution. Large amounts of nutrients may be required; at one site, 16.65 tons of chemicals were added (Minugh, Patry, Keech, and Leek, 1983), while a total of 87 tons of food grade quality chemicals were purchased to clean up another site (Raymond, Jamison, and Hudson, 1976). The bacterial population's response to nutrient addition should be monitored and the nutrients adjusted, as necessary.

Oxygen can be supplied to the aquifer by sparging air into wells with Carborundum diffusers powered by paint sprayer-type compressors (Raymond, Jamison, and Hudson, 1976) or diffusers made from a short piece of DuPont Viaflo tubing for smaller wells (Raymond, Jamison, and Hudson, 1978). The large diffusers can provide up to 10 $ft^3$ of air per minute (scfm) while the smaller tubing diffusers can provide only 1 scfm. Another approach is the use of diffusers spaced along air lines buried in the injection trench (Minugh, Patry, Keech, and Leeks, 1983). The size of the compressor and the number of diffusers are determined by the extent of contamination and the period allowed for treatment (Raymond, 1978). The supply of dissolved oxygen may be the limiting factor in the biostimulation process, especially in low-permeability aquifers (Raymond, Jamison, and Hudson, 1978). As the levels of contamination decrease, the biochemical oxygen demand lessens and the Dissolved Oxygen (DO) level in the water rises. The system must be monitored to ensure that the levels of nutrients are at their optimal concentrations and are being evenly distributed, and that the discharge water meets state or Federal requirements (Raymond, 1978). Adequate supplies of nitrogen and phosphate can be readily maintained once breakthrough has occurred (Raymond, Jamison, and Hudson, 1978).

The process is effective even in aquifers with low permeabilities (Ward and Lee, 1984). A laboratory study showed that gasoline-utilizing bacteria could penetrate sand columns with effective permeabilities ranging from 200 to 3.5 darcys (sand packs of coarse 20-mesh sand to very fine 80+ mesh sand) and consolidated sandstone cores with effective permeabilities of 19 and 75 millidarcys (Raymond, Jamison, and Hudson, 1975). The process has been used to restore aquifers of dolomite (Raymond, Hudson, and Jamison, 1976), a highly permeable sand (Raymond, Jamison, Hudson, Mitchell, and Farmer, 1978), and alluvial fan deposits composed of sand, gravel, cobbles, and some clay and silt (Minugh, Patry, Keech, and Leek, 1983).

The Suntech process has had reasonable success when applied to gasoline spills in the subsurface (Wilson, Leach, Henson, and Jones, 1986). Most sites have implemented appropriate groundwater monitoring programs following cleanup. The overall percent removal of total hydrocarbons using this method has usually ranged from 70 to 80 percent. The Suntech process does not provide for treatment of the material above the water table.

## 1.11 O.H. MATERIALS

O.H. Materials Corp. employs mutant bacteria to treat groundwater contaminated with phenol, as well as acrylonitrile, ethylene glycol, propyl acetate, dichlorobenzene, trichlorobenzene, and methylene chloride (Chowdhury, Parkinson, and Rhein, 1986).

## 1.12 SYBRON BIOCHEMICALS

This company produces mutant bacteria to detoxify soils contaminated with hazardous organics. Sybron hazardous spill programs combine patented BI-CHEM bacteria and technical expertise for fast results at up to 50 percent less than alternate cleanup methods.

A method developed by Sybron Corporation (Patent 4,447,539) uses a mutant of Pseudomonas putida CB-173 (ATCC 31800) (Roberts, Koff, and Karr, 1988). This organism is active at temperatures as low as 1 to 4$^{0}$C and can avail a tremendous cost savings in that a wastewater lagoon need not be heated to normal operating temperatures during the winter months.

## 1.13 FLOW LABORATORIES

This company produces mutant bacteria to detoxify soils contaminated with hazardous organics.

## 1.14 GENERAL ENVIRONMENTAL SCIENCES

This company produces mutant bacteria to detoxify soils contaminated with hazardous organics.

## 1.15 BIOSCIENCE MANAGEMENT, INC. (BMI)

The company can provide feasibility studies, specialized microbial culture development, analytical support, application and process technology, equipment, site monitoring, field supervision, and troubleshooting. BMI has developed a microbe repository from which a symbiotic community of microorganisms may be selected to accelerate the natural decomposition processes. These organisms are used to augment those already present in contaminated surface and subsurface soil and water, in a program of on-site remediation. BMI will provide complete biological cleanup programs, including specialized culture system development and supply, analytical support, biological equipment systems, field supervision, and troubleshooting.

The product, MICROCAT-XBS, is a synergistic blend of preselected, adapted microorganisms for biodegrading oily wastes in water, soil, or sludges. It contains a combination of aerobic and facultative anaerobic microorganisms selected from nature for their ability to break down a broad range of oily substances encountered in wastes from a spectrum of sources. The product contains about $3 \times 10^9$ organisms/g, is packaged in 50-pound fiber drums, and has a shelf-life of one year.

MICROCAT products are a combination of selected activated carbons and specialized microbes. Powdered activated carbon acts as a buffer by adsorbing toxic and inhibitory compounds while providing an inert growth support for the specialized microorganisms. According to the company literature, these products are the least expensive on the market today, for a cost of less than one cent/thousand gallons treated. Table B-2 shows the MICROCAT products available and the industries where they are generally used.

Wild-type strains known to degrade a specific organic chemical or functional group are exposed to successively increasing concentrations of that chemical. The fastest growing strains are isolated and further adapted. Organisms with the highest growth rates are then irradiated. Genetic changes can further increase the growth rate and stabilize the degradative capability.

Roughly, biological methods, as a substitute for incineration, chemical oxidation, encapsulation, and impoundment, and similar waste disposal techniques, are one order of magnitude lower in cost. For example, contaminated soils that might otherwise be landfilled at a cost of approximately \$150 to \$200/$yd^3$ can be biodegraded <u>in situ</u> at \$10 to \$20/$yd^3$.

More than 400 microbial strains are maintained on computer file for easy microbial formulation for a contamination of known composition. If the waste is a complex mixture of both organic and inorganic substances or the composition or biodegradability of the waste is not known, a laboratory investigation is necessary to develop the kinetics of biodegradation of the material. Automated respirometric techniques have been developed to establish:

1. Biodegradation rates (kinetics)

2. Potential for inhibition of these rates at various concentrations

3. Oxygen and nutrient requirements

Table B-2. MICROCAT[R] Product Line

| Product Designation | Product Description | Industry of Use |
|---|---|---|
| HX | Hydrocarbon degrader | Chemical<br>Petroleum refining<br>Pharmaceutical<br>Metals processing<br>Textile |
| RX | Emergency upset additive | Industrial<br>Municipal |
| SX | Degrader of fats, greases, proteins, cellulosics, etc. | Municipal<br>Food processing |
| XP | Degrader of sizes, dyes, cellulosics, lignins | Pulp and paper |
| XNL | Oxidizes ammonia | Industrial<br>Municipal |

4. Temperature effects

Hydrocarbon uptake through the microbe cell wall occurs only with dissolved organic molecules. In the presence of insoluble material, microbes will synthesize and secrete biopolymers in order to first pseudosolubilize the hydrocarbons. Spill biodegradation rates can be enhanced by solubilizing the contaminant through the use of synthetic biodegradable emulsifiers and microbes that secrete large quantities of surface-active biopolymers.

Before treatment begins, it is important to know the amount of material spilled to determine the nutrient requirements for biodegradation. Understanding of the extent and nature of contamination and geology of the site is essential. Biological methods are used in conjunction with physical methods, such as pumping of gross contamination, physical containment, and physical removal. Oxygen is supplied below the soil surface as nutrients, and applied organisms are mixed into the soil by tilling. When deeply buried wastes are contaminating groundwater, the water may be pumped to the surface where aeration and nutrients can be added, and then pumped back into the ground at a point upstream of the groundwater flow.

Mutant microbes are developed to function well at any temperature. Generally, microbial growth rates double for every 20$^{0}$C increase in temperature. Decontamination programs should be undertaken under as warm as possible conditions. Extremes of soil moisture should be avoided and biodecontamination programs avoided at such times. Soil pH should be maintained in the neutral to mildly alkaline range. Aerobic breakdown of organic molecules may cause accumulation of organic acid intermediates that reduce soil pH and inhibit biological activity. These effects can be handled with regular reinoculation and use of chemical pH control agents, such as lime.

## 1.16 POLLUTION CONTROL TECHNOLOGIES

This company produces a series of products that are being used on a commercial basis to biodegrade lignin waste from paper mills, and to break down DDT and PCBs (Holt, 1986). The bacterial products also degrade high levels of waste oil in reserve pits.

## 1.17 DETOX, INC.

### Detox Portable Equipment

Easy-to-install portable organic and inorganic treatment systems can be supplied for groundwater cleanup projects and require no construction. This system needs only interconnecting piping and wiring, comes fully assembled, and can be leased for a project. It is easy to operate and has been proved on full-scale groundwater installations. Groundwater can take from two months to 20 years to clean up, and contaminants may change over this time, requiring a redesign of the treatment system. Detox uses a "Life-cycle Design" that lasts the entire length of the project (see Figure B-3) (Detox, Inc.).

The biological treatment is the least costly method of organic destruction, being 1/20th the cost of carbon adsorption. About 99 percent of all organic compounds can be destroyed by biological reactions. When used in conjunction with other treatment technologies, virtually all of the organics contained in a contaminated groundwater or process stream can be removed and destroyed. An in situ treatment is the best way to clean associated land contamination and the fastest way to clean aquifers. It maintains the surrounding environment while cleaning the contaminated area. Carbon adsorption removes low concentrations of organic contaminants or residual organics from other treatment systems. It is the best system for emergency response. Heavy metal treatment systems range from those that use hydroxide precipitation for metal removal to more complicated systems that remove arsenic from a brine aquifer.

The Detox System comes in several series:

### B-Series

* 5- to 50-ppm organic loading capacity
* 1- to 10-gpm flow capacity/unit
* No operating expertise required
* Minimal operating cost
* Low capital cost
* Quick start-up

This series is intended for short-term projects (three to 12 months) with low flow rates and variable influent concentrations. These reactors consist of a high surface-area "bag" inside of a reactor tank, providing a surface for microbial attachment and filtering the effluent. Oxygen transfer is accomplished through a diffused air system.

### L-Series

* 1- to 30-ppm influent organics
* 1- to 250-gpm flow rate/unit
* Low (1- to 20-ppb) effluent levels
* 1- to 2-day start-up time
* No operator expertise required
* Minimal operation costs

**Life-Cycle Design - Installed system at Southern Texas site (treating phenol in a brine aquifer)**

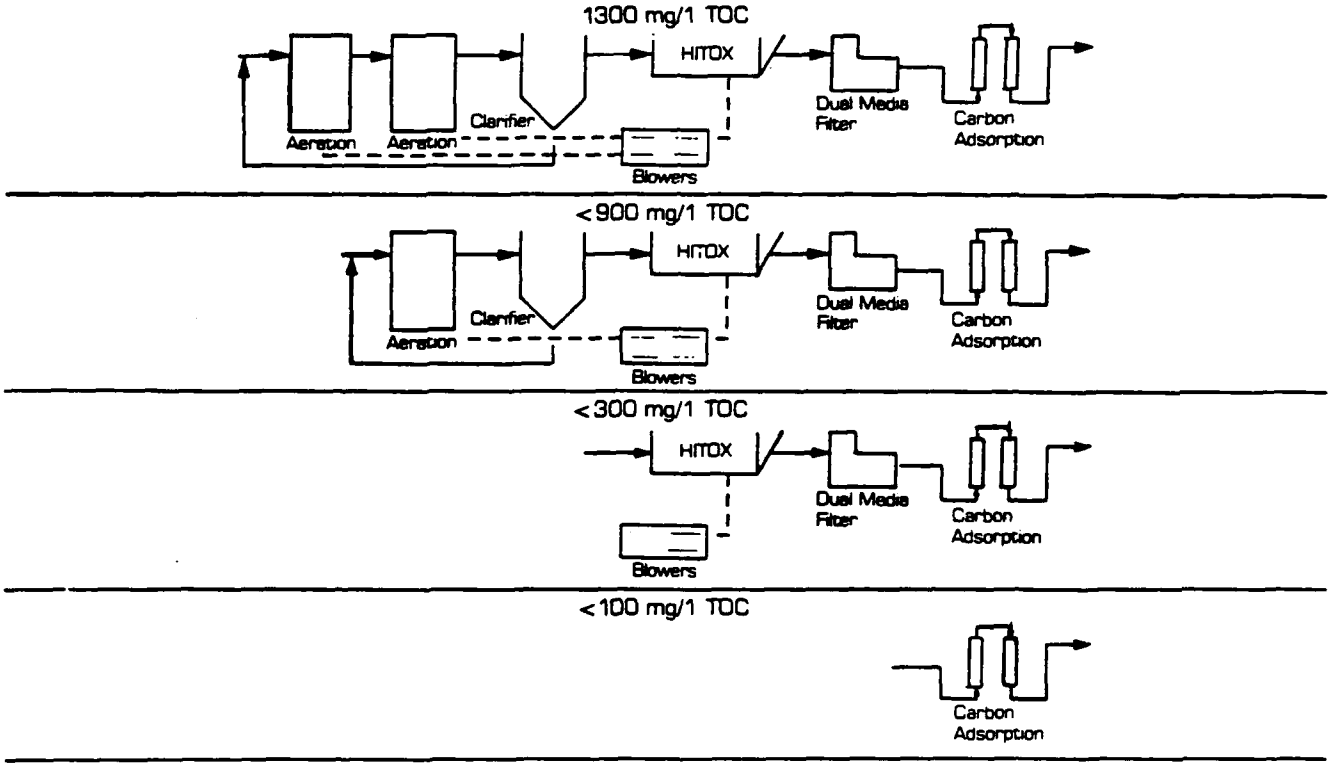


Figure B-3. Life-cycle Design (Detox, Inc.)

These reactors are designed for treatment of low-concentration organic wastes that have to be treated to extremely low effluent concentrations by using biological methods. They are available in standard sizes or can be custom designed.

H-Series

* 50- to 10,000-ppm influent organics
* 2- to 170-gpm flow rate/unit
* Easily operated
* Low operating costs
* Self-regulating
* Custom designed

These custom-designed units contain a submerged, fixed-film medium on which the microbes attach. There is an active transfer of oxygen using diffused air, which also mixes the liquids in the tank. These reactors combine the best elements of activated sludge treatment: low treatment costs and a high degree of treatment efficiency, with the simplicity of operation associated with a fixed-film system. The units are shop assembled for quick, inexpensive installation. They have a maximum capacity of 1000 pounds of organics/day/unit, with a maximum flow rate of 250,000 gal/day.

All DETOX biological reactors can be used in conjunction with in situ treatment programs. They degrade extracted aqueous phase compounds; highly oxygenate the water before returning it to the aquifer; and serve as a source of microbial inocula and a place to control the nutrient addition.

## 1.18 ULTRON

Ultrox International (Culver City, CA) formed an alliance with Keystone Environmental Resources, a subsidiary of Koppers, to expand its presence in groundwater and wastewater treatment in the wood-treatment and coal tar industries (Brooks and McGinty, 1987). Ultrox has developed a packaged unit that uses a combination of ozone or hydrogen peroxide and ultraviolet light to oxidize organics in water, including PCBs, pesticides, and chlorinated solvents. A typical system includes an oxygen or air source, an ozone generator or hydrogen peroxide feed system, a reactor, and an ozone decomposer. The reactor provides the controlled combination of UV and ozone that induces rapid photochemical oxidation of halogenated organic compounds. The system can be operated on a continuous or batch operation. Reactor sizes range from 75 to 6,000 gal. Flow rates can vary from 1 to 1,000 gal/min.

In contrast with air stripping or activated carbon treatment, UV-ozone treatment does not emit toxics to the atmosphere or adsorb them onto media that require landfill disposal or regeneration. Nor does it merely concentrate the toxic compounds, as reverse osmosis does. And, unlike biological treatment, UV-ozone is said to be effective for a wide range of conditions and can be run intermittently.

The system is being use to destroy phenol and pentachlorophenol in groundwater and wastewater. It can also reduce levels of humic acid in groundwater.

## 1.19 AWD TECHNOLOGIES

Dow Chemical joined with Guy F. Atkinson and Woodward-Clyde Consultants to form AWD Technologies (San Francisco), a company that proposes to bring chemical engineering techniques to waste treatment (Brooks and McGinty, 1987).

This company wants to combine the expertise of civil and chemical engineers to develop innovative treatments. An example is the use of Dow gelling agents to slow the movement of polluted groundwater and processing to remove dioxins from water.

## 1.20 WASTE MANAGEMENT AND PURIFICATION

Gamma radiation is the specialty of this company in Plainview, NY, which hopes to use its expertise in tasks as diverse as groundwater cleanup and removal of polychlorinated biphenyls (PCBs).

UV light created during exposure of a fluid to gamma radiation in the presence of ozone will remove the chlorine atoms from complex molecules, like PCBs. It has been used on groundwater containing a number polycyclic aromatic hydrocarbons. Treatment dropped the total organic carbon (TOC) content from 36 to 8 ppm.

## 1.21 ENVIRONMENTAL SOLUTIONS

Waste Management and Purification has formed a partnership, Environmental Solutions, that proposes to build and operate a fluid irradiator to detoxify PCB-contaminated transformer fluid (Brooks and McGinty, 1987). The source of radiation would be cesium-137, a waste material generated by the nuclear power industry. The cesium-137 is contained within a concrete block with all the plumbing on the outside. There are no moving parts, and the system requires no energy to operate.

Excavation costs between $1.75 to 4.50 per cubic yard ($2.30 to 5.90 per cubic meter) plus additional costs for transportation of the contaminated material and disposal in an approved facility.

## 1.22 CALGON CARBON CORPORATION

Granular activated carbon (GAC) is a frequently adopted treatment for contamination episodes involving organic chemicals (Josephson, 1983a). GAC systems have been installed at 31 sites in the U.S. to treat contaminated groundwater flows of 5 to 2250 gpm. In some cases, multimedia filtration was done before GAC treatment as a precaution; in others, air stripping was used to reduce the amount of volatile organics. Influent concentrations of carbon tetrachloride have been reduced from 130 to 10,000 ug/l to below 1 ug/l, and trichloroethylene was reduced from 5 to 16,000 ug/l to less than 1 ug/l. Operating costs are from $0.22 to $2.52/1000 gal treated, depending upon the chemicals and their concentrations.

## 1.23 ZIMPRO, INC.

This company is simultaneously using microbes and an organics removal approach involving powdered activated carbon (PAC) with activated sludge (Josephson, 1983a). The PAC adsorbs contaminants that the microbes cannot assimilate, while the microbes handle organics that otherwise would need an additional carbon step. Its units, presently in use at 15 U.S. sites, can remove hydrocarbons, pesticides, and certain other organics from groundwater, wastewater, or leachates, with a 95 to 99 percent removal efficiency reported for COD, total nitrogen, and various organics. The system, called "PACT," is being used in a groundwater recharge project in El Paso, TX, and for groundwater treatment at a chemical plant site in Michigan.

## 1.24 SOLMAR CORPORATION

This company offers bioculture formulations. These are supplied in dry form and can be fully activated by presoaking for 4 to 12 hr in lukewarm (80° to 100°F) water. Two gallons of either tap water or wastewater are used for each pound of cultures formulation. After presoaking, the cultures are added as far upstream as practical and before primary clarification. For lagoon applications, the slurry is applied over the surface of the entire lagoon. Specific programs are tailored to individual sites.

The formulations are based upon harmless saprophytic, soil-type microorganisms that utilize nonliving organic matter as their food source.

For greatest effectiveness, a system should be treated at pH 7. Formulations may usually be used in the range of pH 5.5 to 8.5. The cultures are mesophilic strains, which operate in the range of 55° to 105°F.

## 1.25 CRANFIELD INSTITUTE OF TECHNOLOGY

A patent has been assigned to a researcher at Cranfield Institute of Technology in England for methane-utilizing bacteria adapted to using methanol as a carbon source to oxidize alkanes and alkenes (Roberts, Koff, and Karr, 1988). Methylosinus trichosporum and Methylococcus capsulata are used for the oxidation of benzene to phenol, propylene to propylene oxide, and toluene to benzoic acid and p-hydroxytoluene.

## 1.26 CETUS COMPANY

The Cetus Company developed a novel multienzyme process for the oxidation of propylene (Hou, 1982). The first enzyme reaction converts olefin to halohydrin in the presence of halide, hydrogen peroxide, and haloperoxidase. The latter can be obtained from horseradish, seaweed, or Caldariomyces. In the second reaction, propylene halohydrin is transformed to propylene oxide by halohydrin epoxidase or by whole cells of Flavobacterium sp.

## 1.27 INDUSTRIAL MICROGENICS, LTD.

This company markets a bacterial strain (Aro-Go) for degrading chlorinated phenols, phenoxylated alcohols, acrolein, and acrylonitrile.

## 1.28 FLORIDA STATE UNIVERSITY

A collection of isolates from the Savannah River deep subsurface is being maintained at Florida State University in Tallahassee (Fredrickson and Hicks, 1987). Several thousand cultures obtained from depths of up to 300 m are being preserved in cryoprotectants, such as dimethyl sulfoxide, at -90°C. These stock cultures will be available to qualified investigators whose proposals are approved after peer review by DOE. Selected isolates are being used for the production of biomarkers by use of fluorescent antibodies to determine the autoecology of specific deep subsurface bacteria.

# APPENDIX C

## COMMERCIAL PRODUCTS, EQUIPMENT, PROCEDURES, AND COSTS IN BIORECLAMATION

## DISCLAIMER

This appendix presents a sample of the types of products, equipment, and procedures being used for bioremediation and their associated costs. This is, of necessity, only a partial list of what is actually on the market. This information is intended to illustrate to the reader the range of materials and procedures employed in biodegradation. It was derived from papers available to the author at the time the report was written in 1988. Reference herein to any specific commercial product, equipment, procedure, process, or service does not constitute or imply its endorsement, recommendation, or approval.

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## SECTION 1

## PRODUCTS, EQUIPMENT, PROCEDURES, AND COSTS

Cost and form of treatment are the most notable engineering problems in dealing with hazardous waste-contaminated sites (Atlas, 1977). Pollution treatment systems are expensive, and scientific principles must be used to develop the most effective low-cost system. This appendix presents some of the products and equipment required for various remediation technologies and, where possible, the costs associated with them. This will allow the expense of a particular remediation effort to be calculated and a comparison to be made of the relative costs of the different treatment options available.

Costs for biological in situ treatment are determined by the nature of the site geology and geohydrology, the extent of contamination, the kinds and concentrations of contaminants, and the amount of groundwater and soil requiring treatment (Environmental Protection Agency, 1985b). There is no easy formula for predicting costs. Costs provided for actual site cleanups indicate that biological treatment can be far more economical as an alternative to, or in conjunction with, excavation and removal or conventional pump and treat methods.

The time and cost of an enhanced bioreclamation project is significantly affected by the amount of nutrients needed to degrade the contaminant, and by the rate at which the nutrient solution can be introduced into the formation (Brubaker and O'Neill, 1982). Thus, an analysis of the total soil and water contamination and an estimate of sustainable pumping rates through the site become important in predicting project cost. This is not to imply that the technology is excluded from formations with low permeability, but the time and cost will be affected, if large amount of degradable contaminants are present.

Estimating the total reservoir of contamination in the soil is an important factor in predicting the time and cost of all pump and treat techniques, as well, even though this estimation is not always made (Brubaker and O'Neill, 1982). This issue often leads to somewhat of a dilemma, however, as the cost of obtaining this information can be quite high, and it has little value beyond allowing a better estimate of how long the remediation process will be required.

The biological treatment is the least costly method of organic destruction, being 1/20th the cost of carbon adsorption. About 99 percent of all organic compounds can be destroyed by biological reactions. When used in conjunction with other treatment technologies, virtually all of the organics contained in a contaminated groundwater or process stream can be removed and destroyed. An in situ treatment is the best way to clean associated land contamination and the fastest way to clean aquifers. It maintains the surrounding environment while cleaning the contaminated area. Carbon adsorption removes low concentrations of organic contaminants or residual organics from other treatment systems. It is the best system for emergency response. For heavy metal treatment, systems can be installed that use hydroxide precipitation for metal removal to more complicated systems that remove arsenic from a brine aquifer.

## 1.1 EXAMPLES OF COSTS INCURRED IN BIORECLAMATION

* Decontamination based upon incineration or wet chemical treatment of excavated soil is costly, involving capital costs of at least $10,000,000 and operating costs of over $3,500,000/yr (Arthur D. Little, Inc., 1976).

* The total cost of the site demonstration at Eglin Air Force Base is $780,000 (Sanning and Olfenbuttel, 1987). Of this total, approximately 50 percent is for design and operation of the system; 25 percent is for sampling and analysis; 15 percent is for nutrients and hydrogen peroxide; and 10 percent is for well installation and delivery equipment. The study will be conducted over two years to provide information on the lower limits of biological removal of fuel compounds. A control area has been established in a fuel-contaminated area on the upgradient end of the site, in order to compare natural levels of degradation with the enhancement provided by nutrient/oxygen additions.

* Total capital and research and development costs for cleanup of the Biocraft site were $926,158, including $446,280 spent on process development (R&D) (1981 figures). Project costs also included the hydrogeological study, and design and operation of the groundwater injection and collection system, and biostimulation plant. Total operating costs, based upon treating 13,680 gal/day, were approximately $226/day, or $0.0165/gal. The total cost, including amortization based upon projected costs, is $0.0358/gal over a three-year period. Prior to the biological treatment program, contaminated water had been removed at a rate of 10,000 gal/month. The average disposal cost had been $0.35/gal. The cost of biological treatment of an equal number of gallons is an order of magnitude less than that for disposal. The Biocraft site employed surface biological reactors, as well as enhancing in situ treatment by reinfiltrating oxygen and nutrient-treated groundwater. Cost for in situ treatment alone would have been less because process plant design and equipment would not be included in an in situ approach.

* The following are the estimated site cleanup costs for hypothetical sites involving the use of hydrogen peroxide as an oxygen source for the enhancement of in situ biodegradation (see also Figure C-14) (Environmental Protection Agency, 1985b). The cleanup of 300 gal of gasoline from a sand gravel aquifer over a period of six to nine months is between $70,000 and 120,000. Cleanup of 3,000 gal of diesel fuel from a fractured bedrock formation is estimated to require nine to 12 months and $160,000 to 250,000. The cost estimate for degrading 10,000 gal of jet fuel from a fine gravel formation is estimated to cost $400,000 to 600,000 and take 14 to 18 months.

* Major costs of oil waste disposal by soil cultivation include fertilizer material and labor for spreading fertilizer and for cultivating the contaminated soil (Kincannon, 1972). These costs were translated to $7.00/barrel, with a disposal rate of 70 barrels/acre.

* Excavation involves removal and transport of contaminated soil to a secure site, such as a landfill; groundwater is usually pumped out and treated (Lee, Wilson, and Ward, 1987). Excavation is usually accomplished by a dragline that can reach a maximum depth of 60 feet (18.3 m) or a backhoe that can go to 70 feet (21.3 m) (Ehrenfeld and Bass, 1984). Excavation costs between $1.75 and $4.50 per cubic yard ($2.30 to $5.90 per cubic meter) plus additional costs for

transportation of the contaminated material and disposal in an approved facility.

* In situ destruction of the phenolic compounds in the soil environment using biological treatment techniques provided an estimated cost benefit of $70,000 (a 30 percent savings) when compared with the cost of secure landfill disposal (Flathman and Caplan, 1985). This was based upon estimated transportation and disposal costs of $230,000 and the actual job cost of $160,000. With biological treatment of greater soil volumes, proportionally greater cost benefits would be realized. In addition to the cost benefit achieved, future liability was minimized, as a result of on-site destruction of the phenolics in the soil environment.

* The costs for surface caps of an impermeable material, such as clay or cement, for contaminated zones range from $20,000 to 30,000 per acre (Lee and Ward, 1986). Liners that prevent leachate from leaving the site can range from plastic to concrete, asphalt, and clay and are estimated to cost from $40,000 to 50,000 per acre.

* A cost estimate for a granular activated carbon treatment system for 100,000 gpd of a wastewater containing 1,000 ppm phenol was a $1.9 million initial investment with a unit cost per 1,000 gal of wastewater of $29.83 (Ehrenfeld and Bass, 1984).

* Operating costs for another granular activated carbon system (Calgon Carbon Corporation) are from $0.22 to $2.52/1000 gal treated, depending upon the chemicals and their concentrations (Josephson, 1983a).

* Expenses for traditional cleanup techniques for marine oil spills have been estimated to average $6.50/gal of spilled oil (Bartha and Atlas, 1977). This translates to roughly $2000 per metric ton, leading in case of major oil spills to multimillion dollar expenses for cleanup alone.

* Cell-free enzymes for treating hazardous waste constituents are not currently in bulk production. Only eight companies accounted for 90 percent of worldwide production of industrial enzymes in 1981; five of the firms are in Western Europe. Only 16 enzymes (primarily amylases, proteases, oxidases, and isomerases) accounted for 99 percent of the 1981 market. This suggests that specialized enzyme production, even on a large scale, may be quite expensive. Current prices for bulk enzyme materials range from $1.45 to $164 per pound. If the enzyme can be produced through chemical synthesis, it will be much less expensive than if it is produced by microorganisms in fermenters.

* Different mixtures are formulated by the company, Interbio, to digest specific types of waste. One such product, called Biolyte, is being used for treating municipal and industrial wastewater. End prices depend upon the level of service provided with the product, but will be around $20/lb. Another product from this company is Biofree, an institutional cleanser that digests grease. It sells for $28/28-oz case (Cooke and Bluestone, 1986).

* Since the oily sludge generation rates at Naval facilities are fairly low (200 to 2000 gpd), the size of an activated sludge treatment system could be relatively small (Roberts, Koff, and Karr, 1988). EPA projections for a system capable of treating 100,000 gpd indicate a construction cost of $78,500, with

annual O&M costs of $4,300. This design assumes oil and grease concentrations of less than 0.1 percent, rather than the 1 to 10 percent oil present in Navy sludge. At least one manufacturer (Envirex) is of the opinion that the rheology of oily sludge makes it unsuitable for treatment in the Rotating Biological Contactor (RBC).

* Plant material, such as alfalfa meal, has been shown to stimulate biodegradation (Arthur D. Little, Inc., 1976). Eight tons/acre of alfalfa meal is equally as effective in stimulating microorganisms as 80 tons/acre of cattle manure. Alfalfa meal can be obtained from commercial animal feed manufacturers for a fee of approximately $5.00 per 100-lb bag. Assuming that about 8 tons are required per acre, the cost of the plant material itself will be about $800 per acre. Transporting the material by truck over a distance of, for example, 20 miles would cost about $2.00 per ton. The plant material would have to be spread over the area at a cost of about $3 to $5 per acre, followed by a discing operation at a cost of $1.50 per acre. The total cost, therefore, of adding the plant material would equal about $820.00 per acre.

* Diking is a common agricultural practice (Arthur D. Little, Inc., 1976). A tool designed to do this is the disc ridger, a bordering tool, which can be purchased for $500 to 600. The disc ridger is attached to a tractor and operated by one man, diking about 2 acres/hr, using available soil. Assuming a reasonable even land, plots could range from 1/4 to 1/2 acre in size. An irrigation system could supply the water. Figuring a diking cost of about $6 per acre for 1/2-acre plots, a maintenance cost of about $8 per acre, and an irrigation cost of about $60 per acre, the total cost for maintaining anaerobic conditions would be about $68 per acre.

* The quickest and cheapest way of increasing the oxygen content is to aerate the soil through repeated discing (Arthur D. Little, Inc., 1976). Assuming about 25 discing operations would be required over a treatment period, the cost per acre would be $37.50.

* Black PVC sheeting, 10 mils thick, can be obtained from WaterSaver Co. in Denver at a cost of 6.5 cents per sq ft (Arthur D. Little, Inc., 1976). The sheeting, which comes in a variety of sizes up to 101 ft wide, can be specified for any length. It must be applied with a front-end loader or similar vehicle and six to 10 men. The sheeting, folded in an accordion manner, is pulled off the loader as it proceeds and laid down by men following behind the loader. Ten men can lay about five sheets a day. If the sheets are to be sealed together, it can be done by hand or with a hydraulic sealer, along with a team of three men (the sheets could be weighted down on the corners or held with soil). The cost of the material would equal about $2,831 per acre. The cost of laying the material would be about $30 per acre, with about $5 added for maintenance. After use, the sheeting could be disced into the soil at a cost of $1.50 per acre. The total cost of raising the soil temperature would be about $2,870/acre. Because of this high cost, it is important to conduct field tests to establish the importance of temperature (and ability of sheeting to significantly raise temperature) prior to implementation of any large-scale measures.

* Probably the best method for adding moisture is to use existing irrigation methods (Arthur D. Little, Inc., 1976). Temporary irrigation systems suitable for this program cost about $50 per acre, fixed annual capital cost. Annual

operating costs are about $12 to 15 per acre, and assuming a 2-year operation, this brings the cost to about $62 to 65 per acre.

* Ferrous sulfate can be added to the soil to decrease alkalinity (Arthur D. Little, Inc., 1976). It is available from commercial sources at a cost of about $24/ton, when purchased by the carload (50 tons). Freight cost over a 100-mile distance would be about $5/ton. Assuming that about 5 tons/acre are needed, the cost per acre would be about $145/acre.

* To increase alkalinity, limestone should be added to the soil at a rate dependent upon the particular pH to be attained (Arthur D. Little, Inc., 1976). To increase alkalinity of a soil that has a current pH of about 7 to a pH of 8 or 9 would take around 15 to 25 tons per acre. Since limestone costs about $9 per ton (including the equipment and labor for spreading), and since a discing operation would follow at a cost of $1.50 per acre, the total cost per acre will be approximately $158 to $263 per acre.

* Roughly, biological methods, as a substitute for incineration, chemical oxidation, encapsulation, and impoundment, and similar waste disposal techniques, are one order of magnitude lower in cost (Zitrides, 1983). For example, contaminated soils that might otherwise be landfilled at a cost of approximately $150 to $200/yd$^3$ can be biodegraded in situ at $10 to $20/yd$^3$.

* The cost of soil disposal of oily wastes was estimated at $3.00/bbl. Degradation rates of up to 100 bbl/acre/month were reported, when the oil was applied to fertilized soils (Francke and Clark, 1974).

* Bacterial mixtures, such as Pseudomonas, Nocardia, and Arthrobacter have been used as part of a "cocktail" that may consist of 12 to 20 strains of microbes for treating different types of wastes (Cooke and Bluestone, 1986). The mixture of organisms would depend upon the composition of the waste.

* The following costs are associated with using immobilized cells to treat contaminated groundwater.

Process costs can only be estimated at this time (Roberts, Koff, and Karr, 1988). For example, the cost of treating a paint-stripping waste containing 1500 ppm phenol and 150 ppm methylene chloride in a pilot plant to produce an effluent containing less than 1 ppm phenol and 1 ppm methylene chloride would be as follows:

1. Pilot plant throughput: 175 gph (4200 gpd)
2. Capital costs for a fully automated, skid-mounted pilot plant: $100,000
3. Biocatalyst charge: 1500 pounds
4. Attrition rate of biocatalyst: <1 percent/month
5. One operator per shift at $15/hr, including fringe
6. Operation for 24 hr/day, 340 days/yr

7. Utility requirements:

    a. 2000 std cu ft compressed air/hr at 100 psi

    b. 2 kw electric power

    c. 500 gph cooling water

    d. 250 lb/hr steam at 100 psi

For this pilot-scale operation at 175 gph, the total cost is estimated at between 10 and 15 cents per gallon. Scaling up to 100,000 gal per day would reduce the costs considerably.

The following cost estimates are calculated, based upon the assumption that the capital cost will be between $200,000 and $1,000,000:

1. Plant capacity: 100,000 gpd

2. Biocatalyst charge: 36,000 pounds

3. Attrition rate of biocatalyst: <1 percent/month

4. One operator per shift at $15/hr, with fringe

5. One supervisor at 40 hr/wk

6. Operation for 24 hr/day, 340 days/yr

7. Utility requirements:

    a. Sparging air (20 to 50 psi) - 250 std cu ft/hr

    b. Electric power - 135 kw/hr

    c. Cooling water - 8000 gal/hr

    d. Steam (100 psi) - 3500 lb/hr

The estimated operating costs range between 1.5 and 2 cents per gallon for the range of capital requirement.

## 1.2 TABLES

### 1.2.1 Materials

The following tables provide information on materials that could be used in treating hazardous waste-contaminated sites.

Table C-1. Mulch Materials (Soil Conservation Service, 1979)

| Organic Materials | Quality | Notes |
|---|---|---|
| Small grain straw or tame hay | Undamaged, air-dried threshed straw, free of undesirable weed seed | Spread uniformly--at least 1/4 of ground should be visible to avoid smothering seeding. Anchor either during application or immediately after placement to avoid loss by wind or water. Straw anchored in place is excellent on permanent seedings |
| Corn stalks chopped or shredded | Air dried, shredded into 8" to 12" lengths | Relatively slow to decompose. Resistant to wind blowing. |
| Wood excelsior | Burred wood fibers approximately 4" long | A commercial product packaged in 80-90 lb bales. Apply with power equipment. Tie down, usually. |
| Wood cellulose fiber | Air dried, nontoxic with no growth-inhibiting factors | Must be applied with hydraulic seeder. |
| Compost or manure | Shredded, free of clumps or excessive coarse material | Excellent around shrubs. May create problems with weeds. |
| Wood chips and bark | Air dried, free from objectionable coarse material | Most effective as mulch around ornamentals, etc. Resistant to wind blowing. May require anchoring with netting to prevent washing or floating off. |
| Sawdust | Free from objectionable coarse material | More commonly used as a mulch around ornamentals, etc. Requires anchoring on slopes. Tends to crust and shed water. |
| Pine straw | Air dried. Free of coarse objectionable material | Excellent around plantings. Resistant to wind blowing. |

Table C-1. Mulch Materials (Soil Conservation Service, 1979) (Continued)

| Organic Materials | Quality | Notes |
|---|---|---|
| Asphalt emulsion | Slow setting SS-1 | Use as a film on soil surface for temporary protection without seeding. Requires special equipment to apply. Sheds water. |
| Gravel or crushed stone | | Apply as a mulch around woody plants. May be used on seeded areas subject to foot traffic. (Approx. weight--1 ton/cu yd) |
| Wood excelsior mats | Blanket of excelsior fibers with a net backing on one side | Roll 36" x 30 yards covers 16 1/2 sq. yd. Use without additional mulch. Tie down as specified by manufacturer. |
| Jute, mesh, or net | Woven jute yarn with 3/4" openings | Roll 48" x 75 yd weighs 90 lb and covers 100 sq yd |

Table C-2. Liming Materials (Follett, Murphy, and Donahue, 1981)

| Liming Material | Description | Calcium Carbonate Equivalent | Comments |
|---|---|---|---|
| Limestone, calcitic | $CaCO_3$, 100% purity | 100 | Neutralization value usually between 90-98% because of impurities; pulverized to desired fineness |
| Limestone, dolomitic | 65% $CaCO_3$ + 20% $MgCO_3$, 87% purity | 89 | Pure dolomite (50% $MgCO_3$ and 50% $CaCO_3$) has neutralizing value of 109%; pulverized to desired fineness |
| Limestone, unslaked lime, burned lime, quick lime | CaO, 85% purity | 151 | Manufactured by roasting calcitic limestone; purity depends on purity of raw materials; white powder, difficult to handle--caustic; quick acting; must be mixed with soil or will harden and cake |
| Hydrated lime, slaked lime, builder's lime | $Ca(OH)_2$, 85% purity | 85 | Prepared by hydrating CaO; white powder, caustic, difficult to handle; quick acting |
| Marl | $CaCO_3$, 50% purity | 50 | Soft, unconsolidated deposits of $CaCO_3$, mixed with earth, and usually quite moist |
| Blast furnace slag | $CaSi_2O_3$ | 75-90 | By-product in manufacture of pig iron; usually contains magnesium |
| Waste lime products | Extremely variable in composition | ? | -- |

### 1.2.2 Costs of Products, Equipment, and Procedures

The following tables list costs of products, equipment, and procedures associated with different methods of treating hazardous waste-contaminated sites.

Table C-3. Costs of Portable Treatment Systems (Brooks and McGinty, 1987)

| Unit Type | Cost ($/unit*) |
|---|---|
| *In situ* biological treatment (suspended growth reactor) | $15 to 40/cu yd, treated |
| Rotating biodisks | 0.20 to 1.10 |
| Trickling filter | 0.08 to 0.15 |
| Activated sludge | 0.10 to 0.30 |
| Packed towers | 0.02 to 0.10 |
| Aeration Basins | 0.02 to 0.08 |
| Carbon adsorption | 0.20 to 0.90 |
| Ultraviolet/hydrogen peroxide | 0.04 to 0.18 |
| Belt press | 0.01 to 0.05 |
| Mixing tanks (including chemicals) | 0.03 to 0.29 |
| Equalization tanks | 0.005 to 0.01 |
| Clarifiers | 0.008 to 0.06 |
| Solidification of solids *in situ* | 0.20 to 1.00 |

* All costs per 1,000 gal, except as noted.
Source: Estimated by Geraghty & Miller (Oak Ridge, TN)

Table C-4. Comparative Costs of Various Methods of Treating Soil and Groundwater Contamination (Rich, Bluestone, and Cannon, 1986)

| Method | Scope | Cost ($/cu yd) |
|---|---|---|
| Excavation with off-site landfill (150 miles) | Removes all soil contaminants but does not treat them | 200 to 400 |
| Excavation with off-site incineration | Removes and treats all soil contaminants; needs emission controls | 300 to 550 |
| Excavation with on-site incineration | Removes and treats all soil contaminants; needs emission controls | 130 to 180 |
| Excavation with low-temperature air stripping on-site | Removes and treats all soil contaminants; needs emission controls | 100 to 300 |
| Pumping groundwater to surface, with carbon adsorption or air stripping | Treats only groundwater contaminants; needs carbon disposal or emission controls | 150 to 280 |
| Air stripping *in situ* | Removes volatile contaminants from unsaturated zone, leaving groundwater untreated; needs emission controls | 20 to 25* |
| Bioreclamation *in situ* | Treats soil contaminants in place | 50 to 100 |

* No allowance for emission controls

Table C-5. Commercial Microbial Augmentation Products or Processes Used to Treat Hazardous Waste-contaminated Soils (Sims and Bass, 1984)

| Product Vendor | Product/ Process Name(s) | Description | Treatment | Price $/Unit | Price $/Acre |
|---|---|---|---|---|---|
| Flow Laboratories Environmental Cultures Div Inglewood, CA | DBC Plus; Types A, A-2,B.F, and H-1 | Specific bacteria, freeze dried and air dried | 25 lb/acre | 10.50-15.80/lb | 263-395 |
| General Environment Science Beachwood, OH | LLMO | Mixture of 7 bacterial strains (Bacillus, Pseudomonas, Nitrosomonas, Nitrobacter, Cellulomonas, Aerobacter, Rhodopseudomonas) in liquid suspension | (Site-dependent) | 16/gal | |
| Groundwater Decontamination Systems, Inc. Waldwick, NJ | GDS process | Technique of circulating water from soil into environmentally controlled tank. Nutrients added and water is aerated. Treated water returned to soil. Air may be injected into soil to stimulate further biodegradation. | (Site dependent) | 0.02/ gal treated | |
| Polybac/Cytox Corp. Allentown, PA; San Francisco, CA; Gonzales, FL | Polysoil process | Mutant bacteria mix, N and P fertilizer, and biodegradable emulsifier. | 100 lb organisms + 400 lb fertilizer and emulsifier, if needed. | | 3227-8067/ application * |

Table C-5. Commercial Microbial Augmentation Products or Processes Used to Treat Hazardous Waste-contaminated Soils (Sims and Bass, 1984) (Continued)

| Product Vendor | Product/ Process Name(s) | Description | Treatment | Price $/Unit | Price $/Acre |
|---|---|---|---|---|---|
| | Chemical/ biological augmentation process | Chemical treatment prior to biological to shorten treatment time (currently in experimental and demonstration stages). | | | 40,000-161,300 for total treatment |
| Solmar Corp. | | Freeze-dried bacteria (mostly spore-producing Bacillus spp. | | | |
| Sybron Biochemical, Birmingham, NJ; Salem, VA | Detoxsol | Formulation of mutant bacteria, buffer nutrients, growth stimulator, and detoxifying agents. | 363 lb/ acre | 27/lb | 9,801** |

* Includes labor, equipment, and products. Usually two to six applications required, depending upon degradability with the Polysoil process.

** Prices for treatment of areas larger than 2000 $ft^2$ are negotiable

Source: Utah Water Research Laboratory

Table C-6. Equipment Applicable to Treatment of Hazardous Waste-contaminated Soils (Sims and Bass, 1984)

| Function | Equipment | Principal Use | Examples of Capacity or Size Range | Approx. Cost, $ | Cost Units |
|---|---|---|---|---|---|
| Power implements | Tractors, crawler | May be needed when maximum traction and stability are needed. Especially useful on steep slopes. | Small, 28 maximum drawbar horsepower | 21,800 | each |
| | | | Large, 300 maximum drawbar horsepower | 210,000 | each |
| | Tractors, wheel type, two-wheel drive | Adequate where traction or power requirements are less demanding. | Small, 12 maximum drawbar horsepower | 6,000 | each |
| | | | Large, 164 maximum drawbar horsepower | 66,500 | each |
| | Tractors, wheel type, four-wheel drive | Better traction and higher horsepower available than with two-wheel drive tractors. | Small, 12 maximum drawbar horsepower | 6,800 | each |
| | | | Very large, 552 maximum drawbar horsepower | 275,000 | each |
| Tillers: loosening, aerating, and/or mixing the soil | Plows, chisel | Loosens and aerates soil to 14" depth with minimum vertical mixing. | 10 ft width | 1,500 | each |
| | | | 41 ft width | 18,700 | each |
| | Plows, moldboard | Turn and aerate soil 8 to 12" deep. Poor mixing, but useful in rocky soils. | 3-bottom, 2-way | 4,800 | each |

Table C-6. Equipment Applicable to Treatment of Hazardous Waste-contaminated Soils (Sims and Bass, 1984) (Continued)

| Function | Equipment | Principal Use | Examples of Capacity or Size Range | Approx. Cost, $ | Cost Units |
|---|---|---|---|---|---|
| | Rotary tillers | Effective vertical mixing and aeration of surface 4 to 10" of soil. Combines effects of plowing and cultivation. | 40" width, 15" rotor diameter | 1,330 | each |
| | | | 300" width, 21" rotor diameter | 16,600 | each |
| | Subsoilers, chisel | Break up deep soil with little vertical mixing to 30" or more. | 13 shank, 270" width | 7,640 | each |
| | Subsoiler, double tilling | Turns surface soil with moldboard plow then loosens and mixes subsoil to 20" with a rotary tiller. Fertilizer or other agent can be mixed into the subsoil. | | | |
| | Harrows, disc | Loosen and aerate surface soil. Provide more vertical mixing than most other harrows. | Small, hitch mounted, 450 lb | 450 | each |
| | | | Large, pull type, 16,700 lb | 32,100 | each |
| | Harrows, power | Several varieties of power harrows available that use rotating (vertical or horizontal) oscillating, or reciprocating motion to loosen and aerate surface soil. | Small, 72" width, 10" working depth | 5,000 | each |
| | Harrows, spike | Break up clods formed when plowing sticky soil. Loosen | 7-ft wide section | 160-230 | each |

Table C-6. Equipment Applicable to Treatment of Hazardous Waste-contaminated Soils (Sims and Bass, 1984) (Continued)

| Function | Equipment | Principal Use | Examples of Capacity or Size Range | Approx. Cost, $ | Cost Units |
|---|---|---|---|---|---|
| | | and aerate shallow surface soil. | | | |
| | Harrows, spring tooth | Loosen and aerate shallow surface soil, have vibrating action. | 8-ft width | 940 | each |
| | | | 20-ft width | 2,900 | each |
| Compactors: compacting soil | Rollers | Compact soil surface, improve soil moisture retention, restrict gas diffusion | 4-ft width | 586 | each |
| | | | 16-ft width | 1,620 | each |
| Applicators: application of exogenous agent(s) | Sprayers, hydraulic | Treatment with relatively small amounts of fluid agents; e.g., 20 to 200 gal/acre | Small, hitch mounted, 7.5-ft treatment width | 511 | each |
| | | | Large, hitch mounted, 60-ft treatment width | 9,330 | each |
| | | | Small, self-propelled, 27-ft treatment width | 6,730 | each |
| | | | Large, self-propelled, 70-ft treatment width | 144,000 | each |
| | Spreaders, chemical fertilizer | Apply granular chemical fertilizers or other agents in similar form. Some fertilizer spreaders can be modified to | Small, hitch mounted, 1 to 2 cu ft capacity | 214 | each |

Table C-6. Equipment Applicable to Treatment of Hazardous Waste-contaminated Soils (Sims and Bass, 1984) (Continued)

| Function | Equipment | Principal Use | Examples of Capacity or Size Range | Approx. Cost, $ | Cost Units |
|---|---|---|---|---|---|
| | | apply agricultural limestone. | | | |
| | Spreaders, manure or dried sewage sludge | Apply barnyard manure or dried sewage sludge. | Tractor drawn, 122 cu ft capacity | 2,600 | each |
| | | | Tractor drawn, 391 cu ft capacity | 9,490 | each |
| | | | Truck mounted | Bid | |
| | Spreaders, agricultural limestone | Apply ground lime or dolomite to soil for pH control. | Small, hitch mounted, 12 cu ft capacity | 624 | each |
| | | | Large, truck mounted, 250 cu ft capacity | 15,700 | each |
| | Injectors, liquid | Inject liquid materials below the soil surface. Conventional equipment can inject 1100 gal/min of liquid to 16" below the surface. | Self-propelled | Bid | |
| | | | Tractor-drawn | Bid | |
| Grinders: grind plant materials | Grinders, tub | Grind hay or similar material to be used as organic matter amendment or mulch. | 8 ton/hr | 12,500 | each |
| | | | 15 to 25 ton/hr | 41,000 | each |
| Covers, Mulches Soil coverings and applicators | Plastic sheeting | Cover soil to manage soil temperature or to suppress volatilization | 2 mil thick 10.5 ft wide, 1400 ft long | 90-95 | roll (140 lb) |

Table C-6. Equipment Applicable to Treatment of Hazardous Waste-contaminated Soils (Sims and Bass, 1984) (Continued)

| Function | Equipment | Principal Use | Examples of Capacity or Size Range | Approx. Cost, $ | Cost Units |
|---|---|---|---|---|---|
| | Plastic laying machine | Applies plastic sheeting over the soil by unrolling it, buring, and/or gluing edges; mounted on tractor hitch. | 10.5 ft wide (sizes 9 to 20 ft wide available) | 3,300 | each |
| | Hydro-mulching | Ground plant materials (frequently wood fiber) are sprayed onto the soil in aqueous slurry. Chemical binding may be added to stabilize the material against the wind. | | 1,500-1,700 (Includes mulch, equipment, and labor) | acre |
| Irrigation | Sprinkler, hand move | Apply water to manage soil moisture or soil temperature. Fertilizers or other treatment agent may be applied with the irrigated water. | Portable, 1 to 40 acres | 200-500 | acre |
| | | | Solid set, 1 or more acres | 800-2000 | acre |
| | Sprinkler, self-move | | Motor driven, side roll, 20-80 acres | 300-500 | acre |
| | Sprinkler, self-propelled | | Center pivot driven by water, 40-240 acres | 325-450 | acre |
| | | | Water driven, side roll, 80-160 acres | 275-450 | acre |

Table C-6. Equipment Applicable to Treatment of Hazardous Waste-contaminated Soils (Sims and Bass, 1984) (Continued)

| Function | Equipment | Principal Use | Examples of Capacity or Size Range | Approx. Cost, $ | Cost Units |
|---|---|---|---|---|---|
| Drainage | Perforated pipe drains | Lower shallow water table to improve soil aeration. | Costs depend on depths of drains, soil hydraulic conductivity, and site parameters. | 350-500 | acre |

Source: Utah Water Research Laboratory

Table C-7. Exogenous Agents, Excavation and Hauling Costs (Sims and Bass, 1984)

| Identifying Term | Agent | Approximate Cost ($) | Cost[a] Unit |
|---|---|---|---|
| Acidifying agents | Aluminum sulfate | 235 | ton |
| | Ferrous sulfate | 130 | ton |
| | Ferric sulfate | 108 | ton |
| | Liquid ammonium sulfide | 235 | ton |
| | Sulfur (crude) | 109-126 | ton |
| | Sulfuric acid | 20-96 | ton |
| Activated carbon | Activated carbon, powder | 0.55 | lb |
| | Activated carbon, granular | 1.05 | lb |
| Carbonates/Phosphates (All technical grades) | Calcium carbonate (limestone) | 6.50-35 | ton |
| | Triple super-phosphate | 127-165 | ton |
| Cooling agents | Liquid and gas carbon dioxide | 0.12 | lb |
| | Liquid nitrogen | 0.28 | lb |
| | Solid carbon dioxide | 0.16 | lb |
| | Water ice | 0.03 | lb |
| Excavation and hauling | Excavation | 0.77-4.58[b] (Average c. 2.10) | $yd^3$ |
| | Hauling less than 5 miles | 0.50 | $yd^3$-mile |
| | Hauling more than 5 miles | 0.25-0.30 | $yd^3$-mile |
| Fertilizer (Fertilizer grade) | Ammonia, anhydrous | 135-180 | ton |
| | Ammonia, aqueous | 210 | ton |
| | Ammonium nitrate | 91-115 | ton |
| | Ammonium sulfate | 74-79 | ton |
| | Diammonium phosphate | 165 | ton |
| | Phosphoric acid, 52-54% | 165 | ton |
| | Phosphate, rock | 23 | ton |
| | Potassium nitrate | 277-284 | ton |
| | Sodium nitrate | 130 | ton |
| | Superphosphate, triple | 160-165 | ton |

Table C-7. Exogenous Agents, Excavation and Hauling Costs (Sims and Bass, 1984) (Continued)

| Identifying Term | Agent | Approximate Cost ($) | Cost[a] Unit |
|---|---|---|---|
| | Urea | 200-215 | ton |
| | Blended fertilizers (N,P,K) | | |
| | 16-16-8% | 220 | ton |
| | 16-16-16 | 230 | ton |
| | 18-46-0 | 260 | ton |
| | 29-14-0 | 230 | ton |
| Flushing agents | Caustic soda, liquid, 50% | 150-230 | ton |
| | Citric acid | 0.81 | lb |
| | Hydrochloric acid, 20 Be[c] | 55-115 | ton |
| | Nitric acid, 36°, 38°, 40° Be | 195 | ton |
| | Sodium lauryl sulfate, 30% | 0.29-0.32 | lb |
| | Sulfuric acid | 20-96 | ton |
| Liming Material | Calcium carbonate (Agricultural limestone or dolomite) | 6.50-34 | ton |
| | Lime (85% CaO) | 31.25-32.50 | ton |
| | Hydrated lime (85% CaOH) | 32.50-34.50 | ton |
| Organic Materials | Animal Tankage | 55 | ton |
| | Alfalfa hay | 80-120 | ton |
| | Bone meal, steamed | 300 | ton |
| | Castor pomace | 149 | ton |
| | Cottonseed meal, 41% | 215 | ton |
| | Horse feed (grains and molasses) | 180 | ton |
| | Manure, dairy cattle | 0.1 | $ft^3$ |
| | Peanut meal, 50% | 235 | ton |
| | Sewage sludge, activated | 80 | ton |
| Oxidizing Agents | Hydrogen peroxide, 35-70% | 0.22-0.43 | lb |
| | Ozone generator, 22 lb/day | 40,000-45,000 | each |
| | Potassium permanganate | 2 | Kg |

Table C-7. Exogenous Agents, Excavation and Hauling Costs (Sims and Bass, 1984) (Continued)

| Identifying Term | Agent | Approximate Cost ($) | Cost[a] Unit |
|---|---|---|---|
| Precipitating Agent | Ferrous sulfate | 130 | ton |
| Proton Donors | Methanol | 0.48 | gal |
| | Mineral oil | 2.69-2.72 | gal |
| | Vegetable oils | 0.22-0.47 | lb |
| | Xylene | 1.20-1.60 | gal |
| Reducing Agents | Acetic acid | 0.23 | lb |
| | Iron powder | 1.00 | lb |
| | Soda caustic (NaOH-Tech): | | |
| | Liquid, 50% | 150-230 | ton |
| | Flake, 76% | 500-570 | ton |
| | Granular, 75% | 520 | ton |
| | Sodium borohydride powder | 18-19 | lb |
| | Sodium borohydride stabilized soln, 12% | 16 | lb |
| Resins | Anion exchange | 191-197 | $ft^3$ |
| | Cation exchange | 211-217 | $ft^3$ |
| Soil/Clay | Bentonite, industrial grade | 94 | ton |
| | Kaolin, uncalcined | 58 | ton |
| | Top soil | 4-10 | $yd^3$ |
| Tetren | Tetraethylene-pentamine | 1.70-1.78 | lb |
| Zeolites | Clinoptilolite | 45-50 | ton |

a = Most costs are wholesale, bulk in train car, tank car, or truckload quantities FOB factories or ports.

b = Depends on equipment type and size. Add 60% for hard or rocky soil; deduct 15% in light soil or sand (Godfrey, R.S., ed. 1982. Building construction cost data 1983. Robert Snow Means Co., Inc., Kingston, MA. 421 p.).

c = Be = Baume.

Source: Utah Water Research Laboratory.

Table C-8. Costs of Various Compounds Used for Oxidation (Texas Research Institute, Inc., 1982)

| Compound | Supplier | Cost | Cost/lb $O_2$ |
|---|---|---|---|
| 35% Hydrogen peroxide | PPG<br>Houston | $0.205/lb<br>3500 gal.min. | $ 1.17 |
| Urea peroxide | Robeco Chemical<br>New York | $3.30/lb<br>in 1 ton lots | $18.85 |
| Urea | W.R. Grace<br>Tulsa | $0.117/lb<br>in 1 ton lots | -- |
| Liquid oxygen | Wilson Oxygen<br>Austin | $165 for 4500<br>cu ft | $ 0.41 |
| Compressed oxygen | Wilson Oxygen<br>Austin | $11.45 for 244<br>cu ft | $ 0.67 |

Table C-9. Amounts and Approximate Cost of Oleophilic Fertilizers for the Stimulated Biodegradation of One Metric Ton of Floating Oil (Bartha and Atlas, 1977)

| Compound | Amount (kg) | Cost ($) |
|---|---|---|
| Urea-paraffin adduct (Sun Oil CRNF, controlled-release nitrogen fertilizer) | 73.0 | 80.30 |
| Pyrophosphoric acid dioctyl ester (octylphosphate, Stauffer Chemical Co.) | 8.2 | 8.50 |
| Ferric (2-ethylhexanoate) (ferric octoate, Shephard Chemical Co.) | 0.15 | 0.33 |

Table C-10. Summary of Project Costs--Biocraft Laboratories, Waldwick, NJ (Environmental Protection Agency, 1985b)

| Task | Actual Expenditure, $ | Unit Cost | Period of Performance |
|---|---|---|---|
| Hydrogeological study-Problem definition | 73,948 | -- | 1976 to 1978 |
| In-house process development (R&D) | 446,280 | -- | 1978 to 1981 |
| Groundwater collection/injection system total | 184,243 | -- | |
| 1) Design | (61,490) | | |
| 2) Installation | (122,753) | | 1980 to 1981 |
| Biostimulation plant design and construction total | 221,207 | -- | 1981 |
| 1) Engineering design | (58,400) | -- | 1981 |
| 2) Masonry construction | (73,975) | -- | 1981 |
| 3) Equipment and miscellaneous installation | (88,832) | -- | 1981 |
| CAPITAL AND R&D TOTAL | $926,158 | -- | |
| Operation & maintenance (O&M) | | | |
| 1) Utilities | 47.40/day | | |
| Electricity 26.4 kw (24 hr/day) | (46.82/day) | 0.0739/kwh | 1983 rates |
| Steam 72 pounds (33 kg)/day @ 90 psi | (0.58/day) | 0.008/pound | 1981 |
| 2) Maintenance | 159.93/day | | |
| 3) Nutrient salts | 19.20/day | | 1983 |
| Total water treated - 13,680 gal (51,779 liters)/day | O&M Total: $226.53/day | $0.0165/gal ($0.0044/l) | |

Source: USEPA, 1984

Table C-11. Unit Costs for Installation of a Permeable Treatment Bed (Environmental Protection Agency, 1985b)

| Item | Assumptions | Costs |
|---|---|---|
| Trench excavation | 20-ft deep, 4-ft wide by backhoe | $1.40 cubic yard[a] |
| Spreading | Spread by dozer to grade trench and cover | $1/cubic yard[a] |
| Well-point dewatering | 500-ft header 8" diameter, for one month | $115/linear ft[a] |
| Sheet piling | 20-ft deep, pull and salvage | $7.70/sq ft[a] |
| Walers, connection, struts | 2/3 salvage | $165/ton[b] |
| Liner | 30 mil PVC<br>30 mil CPE | $0.25 to 0.35/sq ft[d]<br>$0.35 to 0.45/sq ft |
| Limestone | Mixed "gravel size" and "sand size" | $30 to 45/ton[c] |
| | Installation (Backfill trench, 100 ft haul) | $3.70/cubic yard[a] |

a = Godfrey, 1984; Costs are total, including contractor overhead and profit.

b = Godfrey, 1984; materials only.

c = Schnell, 1985.

d = Cope, Karpinski, and Steiner, 1984.

Table C-12. Costs Associated with the FMC Aquifer Remediation Systems (ARS) Bio XL Process (FMC Company Sales Literature)

| | Carbon adsorbers | Bioreclamation |
|---|---|---|
| Circulation rate*, $m^3/s$ | $6.308 \times 10^{-3}$ | $6.308 \times 10^{-3}$ |
| Cleanup time, yr | 10 to 20 | 0.33 to 0.66 |
| Fixed costs, $ | | |
| Project evaluation | -- | 90,000 to 70,000 |
| Construction/startup | 120,000 to 450,000 | 50,000 to 75,000 |
| Variable costs, $/yr | | |
| Chemicals | 10,000 | 90,000 to 112,000 |
| Labor | 25,000 | 6,000 to 10,000 |
| Total project cost, $ | 470,000 to 850,000 | 180,000 to 270,000 |

*Influent conc., 20 to 40 ppm

Table C-13. Total Cost for Soil Manipulation (Arthur D. Little, Inc , 1976)

| | $/acre |
|---|---|
| Decrease pH | |
| $FeSO_4$ at $24/ton<br>$5/ton transportation<br>at 5 tons/acre | 144 |
| Increase organic content | |
| Alfalfa meal at $100/ton<br>$2/ton transportation<br>at 8 tons/acre | 820 |
| Decrease oxygen content | |
| Diking at $8/acre<br>Irrigation at $60/acre | 68 |
| Increase temperature | |
| PVC sheeting at $2831/acre<br>Application at $30/acre<br>Maintenance at $5/acre<br>Discing in at $1.50/acre | 2867.50 |
| Increase soil moisture | |
| Fixed annual capital cost for irrigation $50/acre<br>Operating cost $12/acre | 62 |
| Increase oxygen content | |
| 25 discings at $1.50/acre | 37.50 |
| Increase pH | |
| Limestone at $9/ton<br>20 tons/acre<br>Discing at $1.50/acre | 181.50 |
| TOTAL COST | $4180.50/acre |
| 320 acres (1/2-sq mi) | $1,337,760 |

Table C-14. Estimated Costs for Hypothetical Bioreclamations Using Hydrogen Peroxide as an Oxygen Source (Environmental Protection Agency, 1985b).

| | Site A | Site B | Site C |
|---|---|---|---|
| Contaminant | 300 gal gasoline | 2,000 gal diesel fuel | 10,000 gal jet fuel |
| Formation | Sand/gravel | Fractured bed rock | Fine gravel |
| Flow Rate | 50 gpm | 10 gpm | 100 gpm |
| Project Time | 6 to 9 months | 9 to 12 months | 14 to 18 months |
| Estimated Costs | $70 to 120M[a] | $160 to 250M[a] | $400 to 600M[a] |

[a] M = 1000

Source: FMC, 1985

# APPENDIX D

# TESTING METHODS IN BIODEGRADATION

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# SECTION 1

# SCREENING AND ENUMERATION METHODS FOR DEGRADATIVE MICROORGANISMS

## 1.1 GENERAL TECHNIQUES

No place has been found in the United States or Canada--at depths to 400 ft--where sufficient organisms are not present to be brought up in 72 hr to a significant population (Rich, Bluestone, and Cannon, 1986). The bacteria are present; the problem is establishing the right conditions for their growth, in the laboratory, as well as in the field. Media selection is critical in this approach (Thomas, Lee, and Ward, 1985). Growth on plates and enumeration of the organisms has been a standard means of assessing the microbial capability in the subsurface, although metabolic activity of the organisms present would provide more significant data.

The extent of the modification of organic contaminants depends upon biological reactions (Webster, Hampton, Wilson, Ghiorse, and Leach, 1985). In order to be able to predict the fate of pollutants, it is essential to be able to measure the biological activity present in subsurface material. Enumeration of microorganisms can be difficult, since most subsurface bacteria exist in an ecosystem low in organic carbon and do not grow well, if at all, in conventional growth media with high organic carbon concentrations (Wilson, Leach, Henson, and Jones, 1986).

Counting colonies growing on culture media, as was done in the past, is not directly applicable to subsurface microbes that may have unknown growth requirements (Wilson, Leach, Henson, and Jones, 1986). It is difficult to cultivate all of the heterotrophic bacteria present in a soil or water sample on a single medium (Larson and Ventullo, 1983). Nutritional requirements for individual bacteria vary, and even complex nutrient media may not provide essential growth factors for fastidious bacteria. This would make plate count numbers unrealistically low. In addition, many organisms attach firmly to particles (Nannipieri, 1984). Because of aggregation and formation of microcolonies in the environment, the colonies that form on plates may not represent a single viable cell in the sample, which would also lower the count (Larson and Ventullo, 1983).

Different results have been reported from attempts to calculate the number of viable organisms in a sample. Typically, more than 25 percent of the microorganisms isolated will fail to grow on subculture on an artificial medium (Stetzenbach, Kelley, Stetzenbach, and Sinclair, 1985). In other studies, dilution plating techniques with artificial media were capable of detecting only 1 to 10 percent of the total number of soil microorganisms, as determined by microscopic direct counting (Alexander, 1977; Nannipieri, 1984). In yet another investigation, viable cell counts of subsurface samples that were poor in organic material were compared on three different media (Ghiorse and Balkwill, 1985b). A rich organic medium (PYG) had a factor of $10^3$ fewer viable cells than a diluted (5 percent) PYG or a 10 percent soil extract medium. Results suggest that at least 50 percent of the bacteria counted by epifluorescence in these samples were capable of growth. In addition, counts in soil samples taken a few centimeters from each other and even among

subsamples have been found to vary by orders of magnitude (Federle, Dobbins, Thornton-Manning, and Jones, 1986). The huge variation has been attributed to the inadequacies of the enumeration procedures, as well as real heterogeneity of the soils. It should also be recognized that prolonged storage of some core samples may decrease biological activity (Thomas, Lee, and Ward, 1985).

Viable heterotrophs have been enumerated by plating samples on a medium designated TGA (0.75 percent trypticase peptone, 0.25 percent phytone peptone, 0.25 percent NaCl, 0.1 percent unleaded gasoline, 1.5 percent agar) (Horowitz, Sextone, and Atlas, 1978). Gasoline hydrocarbon-utilizing microorganisms have been enumerated on medium BA-G, with incubation at 15°C for one week (Bushnell Haas agar exposed to volatile gasoline hydrocarbons; Horowitz and Atlas, 1977b). Gasoline utilizing microorganisms can be enumerated on medium GA (Bushnell Haas agar with 0.5 percent emulsified leaded MOGAS; Horowitz, Sextone, and Atlas, 1978). Presumptive heterotrophic denitrifiers have been enumerated on Difco nitrate agar incubated at 15°C for one week under an atmosphere of helium.

The media found to be best for enumerating petroleum-degrading microorganisms contains 0.5 percent (vol/vol) oil and 0.003 percent phenol red, with Fungizone added for isolating bacteria and streptomycin and tetracycline added for isolating yeasts and fungi (Walker and Colwell, 1976c). Addition of Fungizone to oil agar no. 2 is selective for actinomycetes (Walker and Colwell, 1975). Washing the inoculum does not improve recovery of petroleum degraders. Specifically, silica gel-oil medium and a yeast medium are recommended for enumeration of petroleum-degrading bacteria and yeasts and fungi, respectively. It is suggested that counts of petroleum degraders be expressed as a percentage of the total population, rather than total numbers of petroleum degraders per se. Normalizing the data, by comparing the percentage of petroleum-degrading bacteria in the total viable, heterotrophic count with the percentage of specific hydrocarbon-extractable material, provides a better estimate of degrading activity. However, there appears to be a "threshold" concentration of oil in the environment or percentage of petroleum-degrading microorganisms in the microbial population of the environment below which there is little correlation between the two. Incubation temperature and presence of oil were found to influence the numbers of petroleum-degrading microorganisms at a given sampling site.

The use of silica gel as a solidifying agent has been shown to improve the reliability of procedures for enumerating hydrocarbon utilizers (Seki, 1976). Others report that a medium containing 0.5 percent oil and 0.003 percent phenol red is best for enumerating petroleum-degrading microorganisms (Walker and Colwell, 1976c). Addition of Amphotericin B permits selective isolation of hydrocarbon-utilizing bacteria, and addition of either streptomycin or tetracycline permits selective isolation of yeasts and fungi. Still others report that plate counts, using either agar or silica gel solidifying agents are unsuitable for enumerating hydrocarbon-utilizing microorganisms, since many marine bacteria grow and produce microcolonies when on small amounts of organic matter (Higashihara, Sato, and Simidu, 1978). Instead, for accurate enumerations of microbial populations that degrade hydrocarbons in marine environments, a most probable number (MPN) procedure was recommended, using hydrocarbons as the source of carbon and trace amounts of yeast extract for necessary growth factors. A miniaturized MPN method has also been developed to

determine the number of total heterotrophic, aliphatic hycrocarbon-degrading, and PAH-degrading microorganisms (Heitkamp and Cerniglia, 1986).

One of the procedures for enumerating specific bacterial populations in environmental samples is the use of selective enrichment techniques (Jain and Sayler, 1987). This technique is based upon the assumption that organisms capable of growth on liquid or agar media containing a pollutant or recalcitrant compound as a sole carbon source must be capable of catabolism of that substrate. This assumption has some serious flaws that affect the utility and reliability of the approach. Selective media prepared for such isolations have usually incorporated the xenobiotic as a primary energy or nutrient source (Kaufman, 1983). In theory, this approach encourage and permits the isolation of all those organisms capable of metabolizing the xenobiotic. In fact, however, it isolates only those microorganisms that are capable of utilizing the xenobiotic as a primary or supplemental source of nutrients and proliferating at the expense of the xenobiotic.

Nevertheless, while these techniques may not be feasible for determining accurate counts, they can be employed for isolating target organisms. The approach generally taken in identifying potential seed organisms is to attempt to isolate hydrocarbonoclastic microorganisms by enrichment culture (ZoBell, 1973). The microorganisms isolated depend largely upon the substrate used in the enrichment, the culture conditions, and the source of the inoculum (Westlake, Jobson, Phillippe, and Cook, 1974). A mineral medium, called Dworkin Foster, is commonly used in studies with hydrocarbon-degrading bacteria and contains the minimal components for growth, except for a source of carbon and energy, such as gasoline (Horowitz and Atlas, 1977b). A low-nutrient medium, R2A, has also been employed for the primary isolation and enumeration of bacteria from well water (Stetzenbach, Sinclair, and Kelley, 1983). Enrichment of well water with low concentrations (100 ug carbon/l or 1000 ug carbon/l) of glucose, acetate, succinate, or pyruvate enhanced the growth of Acinetobacter isolates and an unidentified, oxidase negative, pigmented bacterium.

Many investigators have used n-paraffins for enrichment cultures (Miget, 1973; Atlas and Bartha, 1972c). However, the n-paraffins rarely constitute the major percentage of the compounds found in an oil, and the organisms isolated from these enrichments often do not possess the enzymatic capability to degrade the other classes of hydrocarbon components in petroleum (Kallio, 1975). Use of a crude or refined oil as the substrate is an improvement, but the initial organisms isolated are often those that metabolize the n-paraffins.

Other hydrocarbons have been substituted as the sole carbon source for enrichment cultures (Walker, Austin, and Colwell, 1975; Gibson, 1971). Organisms have, thus, been isolated that can degrade various branched paraffins, as well as aromatic and alicyclic hydrocarbon petroleum components (Gibson, 1971; Dean-Raymond and Bartha, 1975). Sequential enrichment is a modification of this technique (Horowitz, Gutnick, and Rosenberg, 1975). Here, a crude or refined oil or a hydrocarbon mixture is used as the substrate, and organisms are isolated from this first enrichment. The residual hydrocarbons left after the first enrichment usually do not contain n-paraffins and are recovered and used for a second enrichment from which other microorganisms are isolated. Presumably, these organisms are able to attack petroleum components that are relatively difficult to degrade. This sequence can be repeated until

there are no more residual hydrocarbons or until no more organisms can be isolated. A combination of these organisms then will have the enzymatic capability of degrading many different petroleum components. The mixture has been shown to degrade crude oil better than any of the isolates alone.

Bacteria have been the predominant organisms isolated from enrichment experiments in which the soil perfusion technique has been employed (Kaufman, 1983). Soil fungi capable of degrading xenobiotics have been more frequently isolated from enrichment experiments that have used shake-culture techniques. The cultural techniques employed seem to ultimately affect those microorganisms isolated.

The difficulty of applying standard enumeration techniques to environmental samples has led to the use of two other methods for this purpose (Alexander, 1977). One of these is the direct microscopic examination of samples with acridine orange counting (AODC) of the organisms (Ghiorse and Balkwill, 1983; Ghiorse and Balkwill, 1985a). This dye binds to nucleic acids, especially DNA, and is excited with blue light. The method allows bacteria to be distinguished from abiotic particles. When another dye, 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-tetrazolium chloride (INT), is also used, bacteria with active respiratory enzymes will reduce the INT and deposit red-purple INT-formazan granules in their cells, which can also be counted. The proportion of respiring cells then reflects the metabolic activity of a population. Sometimes the intensity of color is difficult to assess; however, if the weakly positive cells are even marginally metabolically active, they would be significant in decomposition of a pollutant (Webster, Hampton, Wilson, Ghiorse, and Leach, 1985).

Another counting method uses a biochemical indicator, such as adenosine 5'-triphosphate, to determine the biomass, or amount of living material present (Webster, Hampton, Wilson, Ghiorse, and Leach, 1985; Hampton, Webster, and Leach, 1983). This technique is involved and requires extraction of the chemical with a mixture composed of $H_3PO_4$, EDTA, adenosine, urea, DMSO, and Zwittergent 3,10, followed by sensitive and specific analysis. A recovery of 98 percent of the ATP has been obtained with the method. The amount of ATP in bacteria during exponential growth is fairly constant. However, when bacteria are exposed to extreme environmental conditions, there can be a wide variation in ATP content (as much as thirtyfold). This can affect the cell count.

An alternate approach is to determine biomass by analyzing the phospholipids extractable from soil (Nannipieri, 1984). Determination of biomass through analysis of the extractable lipids avoids many of the above problems (Federle, Dobbins, Thornton-Manning, and Jones, 1986). Estimates of biomass are not dependent upon growth of the organisms and are not biased by the germination of inactive forms of the microbes, such as spores. They are made on a large sample and are not hindered by the problem of differentiating living and dead cells. This method has been used to estimate microbial biomass in estuarine and marine environments (Gillan, 1983; White, 1983) and in subsurface soils (Federle, Dobbins, Thornton-Manning, and Jones, 1986).

Two other sensitive, accurate, and highly specific detection and monitoring techniques are the use of immunofluorescence microscopy and DNA probes, respectively, which can contribute to quantification of the persistence of genetically engineered microorganisms (Jain and Sayler, 1987).

Immunofluorescence microscopy, which is based upon an interaction between an antibody and its corresponding antigen, has still not been widely used for environmental samples. However, the technique has recently been used to determine survival of E. coli cells suspended in seawater and showed the sensitivity of this method over plate counts (Grimes and Colwell, 1986).

Nucleic acid hybridization or DNA probe techniques (direct detection of specific DNA in the organism) are already proving useful and sensitive for detecting and monitoring the critical populations recovered from the environment; for example, in the enumeration of toluene- and naphthalene-degradative populations in environmental microcosms contaminated with synthetic oils (Sayler, Harris, Pettigrew, Pacia, Breen, and Sirotken, 1986) and in the rapid isolation of organisms capable of degrading polychlorinated biphenyls (Pettigrew and Sayler, 1986).

Probe technology can even detect a single colony containing target genes among $10^6$ colonies from an environmental community (Sayler, Shields, Tedford, Breen, Hooper, Sirotkin, and Davis, 1985). Use of specific chromosomal or plasmid DNA probes to monitor the maintenance of ABS10, AHS24, AOS23, and P. putida (TOL and RK2) inoculated into a groundwater microcosm showed that regardless of the presence of chemical pollutants or selective pressure (toluene, chlorobenzene, and styrene), these organisms were maintained at approximately $1 \times 10^5$ positive hybrid colonies/g of aquifer microcosm material throughout an eight-week incubation period (Jain and Sayler, 1987). Use of specific naphthalene-degrading DNA probes to determine the naphthalene-degrading population in a complex biological wastewater system (a completely mixed aerobic reactor) demonstrated the significance and sensitivity of this technology.

A technique was developed to measure both mineralization and uptake of radiolabeled substrates into biomass, as well as a mass balance of added label (Long, Dobbins, Aelion, and Pfaender, 1986; Dobbins, Aelion, Long, and Pfaender, 1986). [$^{14}$C]hydrocarbons can be utilized as a means of estimating the hydrocarbon-degrading potential of bacteria in estuarine and marine environments. This technique allows assessment of the metabolic activity of subsurface microbial communities, and use of several concentrations permits calculation of metabolic kinetics. The amount of mineralization of [$^{14}$C]hexadecane, for instance, can be equated with the total number of petroleum-degrading bacteria and the percentage of the total heterotrophic population, which they represent. A community can be adapted to metabolize at concentrations significantly higher than have been suggested to exist in its environment. The technique works well for substrates of various polarity and volatility. $^{14}$C-radiolabeled hydrocarbons have also been used in MPN procedures (Atlas, 1978b). The method uses the conversion of the radiolabeled compound to radiolabeled carbon dioxide to establish positive results in the MPN procedure.

Other researchers found that using respiration rates for Most Probable Number (MPN) data did not work well, since respiration was slight for most of the enobiotics (Swindoll, Aelion, Dobbins, Jiang, Long, and Pfaender, 1988). Therefore, this particular method may not be appropriate for this type of compound. Also, measuring only mineralization to estimate the amount of substrate metabolized may produce erroneous conclusions, as seen in the differences in $^{14}CO_2$ evolved for different compounds.

## 1.2 SPECIFIC TECHNIQUES FOR FUNGI AND BACTERIA

A technique using solid agar has been developed to allow rapid analysis of a large number of individual strains or mixtures of fungi for those that grow well on a given hydrocarbon (Nyns, Brand, and Wiaux, 1968). It can also be used to increase the ability of a wild strain to assimilate a hydrocarbon by subculturing of resistant colonies. This method has been varied slightly to determine the ability of fungi to grow on crude oils and single hydrocarbons by substituting another medium (Davies and Westlake, 1979). Slants are inoculated with spores. When mycelia appear, crude oil or n-tetradecane is pipetted halfway up the agar slope. Napthalene, sterilized by UV irradiation, is sprinkled over inoculated plates, which are then incubated in air. Toluene is supplied in the vapor phase by incubating inoculated plates in a closed system containing air and toluene.

Oil-utilizing fungi can be isolated by adding soil to a liquid medium, washing mold colonies that develop on the surface of the enrichment medium, and transferring them to plates of Cooke's aureomycin-rose bengal medium (Cooke, 1973). Yeast colonies are then streaked on 2 percent malt agar. Molds are maintained on slants of mixed cereal agar and yeasts on yeast-malt agar (Wickerham, 1951).

Hydrocarbonoclastic yeasts have been isolated by spreading oil-impregnated waters directly onto an isolation agar medium containing 0.7 percent yeast-nitrogen base and 0.5 percent chloramphenicol (Ahearn, Meyers, and Standard, 1971). The defined yeast-nitrogen base medium of Wickerham (Wickerham, 1951) was employed in assimilation studies.

Soil suspensions are plated onto R2A medium (Reasoner and Geldreich, 1985) and incubated at the average in situ soil temperature of 11°C for at least seven days (Cerniglia, Gibson, and Baalen, 1980b). Representative colonies are restreaked onto R2A agar for isolation of pure cultures. Different mixtures of organisms could be isolated from soils, if the enrichments were carried out at 4° rather than 20°C (Schwendinger, 1968).

To determine counts of JP-5-utilizing bacteria, 0.1 ml of well water, or a dilution thereof, was spread over the surface of a sterile plate of mineral salts agar, which was then inverted over a piece of JP-5-saturated filter paper contained in the petri dish lid and incubated at ambient conditions (18 to 22°C) for seven days (Ehrlich, Schroeder, and Martin, 1985).

Methanogenic bacteria can be determined by multiple-tube procedures, according to the method of Godsy (Godsy, 1980). Sulfate-reducing bacteria can be determined by multiple-tube procedures using American Petroleum Institute (API) broth (Difco, Detroit, Mich.) (Ehrlich, Schroeder, and Martin, 1985). Heterotrophic anaerobic bacteria can be determined by multiple-tube techniques using prereduced, anaerobically sterilized, peptone-yeast extract glucose broth (Holdeman and Moore, 1972).

$^{3}H$ thymidine incorporation can be used to measure growth rates of bacteria in subsurface soils (Thorn and Ventullo, 1986).

## SECTION 2

## OTHER TECHNIQUES IN BIODEGRADATION

Biodegradation can be measured by using either specific analytical or radiochemical techniques (Larson and Ventullo, 1983). Both of these methods allow biodegradation be be measured in complex environmental matrices at realistic (ug/l) environmental concentrations. Radiochemical procedures are especially gaining increased acceptance for use in environmental fate studies. The use of certain isotopes, such as carbon-14 allows the actual metabolism of the compound to be measured. Whereas analytical procedures follow disappearance of the parent compound, studies with $^{14}C$-labeled materials allow the complete (ultimate) biodegradation to $^{14}CO_2$ to be measured. If cumulative $^{14}CO_2$ production is measured as a function of time, both the rate and extent of ultimate biodegradation can also be quantitated. Use of $^{14}C$-uniformly labeled compounds and the turnover time-tracer approach will permit measurement of heterotrophic activity (Azam and Holm-Hansen, 1973; Gocke, 1977). This can be used in the form of a $^{14}C$-radiorespirometric most probable number (MPN) technique (Lehmicke, Williams, and Crawford, 1979).

A microcalorimeter was developed by the Department of Biological and Agricultural Engineering to offer a convenient and relatively rapid way of determining whether a contaminant is metabolizable and what the maximum level of concentration of the contaminant can be (Scholze, Wu, Smith, Bandy, and Basilico, 1986). The microbial activity in terms of heat output for a 1 g sample is a "thermogram." A normalization test performed on a sample that is the organic medium to sustain the microbiological community will reflect the indigenous activity of the microorganisms present. The heat output will be proportional to their numerical density. A baseline thermogram will give background activity in the soil. The area between the baseline and a normalization thermogram is a quantitative measure of the heat contributed by the organisms. The effect of adding a contaminant to the organisms will show whether the compound is toxic (lower heat output) or can be metabolized (greater heat flux than for the normalized thermogram). This is the degradation response. The results will show the feasibility of using the microbiological community to degrade an organic contaminant.

Volatile compounds have been identified as the most frequent and most concentrated contaminants in groundwater (Plumb, 1985). Since it may not be economically feasible to analyze all samples for all possible contaminants, it is suggested that the occurrence of volatile compounds may be a useful screening technique to establish the level of organic analysis required. Only if the occurrence of volatiles were to exceed some predetermined action limit, would more complete organic characterization of samples be justified.

Respiration rates are slight for most xenobiotics (Swindoll, Aelion, Dobbins, Jiang, Long, and Pfaender, 1988). Therefore, this particular method may not be appropriate for this type of compound. Measuring only mineralization to estimate the amount of substrate metabolized may also produce erroneous conclusions, as seen in the differences in $^{14}CO_2$ evolved for different compounds.

Denitrification in sediments can be examined using the acetylene blockage of $N_2O$ reduction technique (Balderston, Sherr, and Payne, 1976). Nitrogen fixation can be estimated by the acetylene reduction method (Hardy, Burns, and Holsten, 1973).

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# APPENDIX E

# BIORECLAMATION ACTIVITIES

# SECTION 1

# INTRODUCTION

Remedial activities will undergo a stepwise development: 1) laboratory studies to verify the appropriateness of employing biodegradative techniques to the remediation program and to establish the factors that need to be manipulated for optimum degradation, 2) pilot tests of the proposed plan on a small scale (in the laboratory or in the field), and 3) field tests at a contaminated site. A pilot test may be a field test. This appendix describes these different stages and in Section 2, Case Histories, presents examples of their application in the development of a bioreclamation program.

The results obtained from laboratory investigations (i.e., those involving enrichment cultures, pure cultures, or cell-free extracts) are important (Berry, Francis, and Bollag, 1987). However, attempts to predict the environmental fate of any organic compound based upon laboratory results alone should be done cautiously. Whenever possible, results from laboratory investigations should be critically correlated with results obtained from in situ investigations. This combination of information should provide a framework for predicting the fate of organic compounds in natural environments.

## SECTION 2

## LABORATORY/MICROCOSM STUDIES

Differences in the physical, chemical, and microbial characteristics of ecosystems can affect the disposition and persistence of chemicals in the environment (Heitkamp, Freeman, and Cerniglia, 1987). Consideration of these differences, as well as the complexity of natural ecosystems and the physical and chemical properties of toxicants, has led to the use of multicomponent microcosms as laboratory models to predict the toxic effects and degradation of chemicals in the environment (Giesy, 1980).

Laboratory (bench-scale) and pilot-scale testing may be necessary to evaluate the technical and practical feasibility of in-place treatment methods for contaminated soil prior to full-scale implementation (Sims and Bass, 1984). Testing may be used to establish the following at in-place treatment sites:

1. The critical soil level for the waste at which treatment (degradation, detoxification, or immobilization) is ineffective due to toxicity or mass flow conditions.

2. The rate of degradation or detoxification of organic constituents, i.e., the half-life, and the extent of immobilization of inorganic constituents.

3. The mobility, toxicity, and biodegradability of partially degraded waste constituents or waste fractions.

4. Criteria for management of the soil and site to enhance the natural ability of the soil to attenuate constituents by determining optimum conditions for degradation, detoxification, or immobilization.

5. Parameters and constituents that should be monitored to indicate contaminant migration to receiver systems, including groundwater, surface water, and atmosphere.

6. Technical feasibility and potential costs associated with using techniques based upon management of the site/soil assimilative capacity for accomplishing the treatment required for a particular site/soil/waste system.

Management of the soil/site system contaminated with organic chemical wastes will help increase the rate of biodegradation (Sims and Bass, 1984). Part of site characterization involves determination of the presence or absence of microorganisms (e.g., aerobic bacteria, anaerobic bacteria, actinomycetes, fungi, and algae) for representative areas of contamination. The ability of these organisms to degrade petroleum hydrocarbons should also be determined. Part of the waste characterization involves analyses to determine the carbon:nitrogen:phosphorus (CNP) ratio. If the C:N:P ratio is not optimal for microbial growth, bench-scale experiments should be conducted to determine whether adjustment of this ratio will increase the rate of biodegradation. These initial experiments should be performed with samples of contaminated

soils from the site at dissolved oxygen concentration levels characteristic of the respective contaminated areas, if possible.

Experiments should also determine the effect of various oxygen levels on rate of biodegradation. If nutrient and oxygen adjustments are ineffective, studies could look at the inoculation of the soil/waste mixture with activated sludge, commercially developed microorganisms that may target the particular contaminants, or soil from the site or control area that has a high microbial count. Rates of biodegradation should be measured systematically as a function of nutrient concentrations and oxygen to establish optimum conditions for degradation.

The rate of disappearance of the parent compound should be determined, as well as the chemical identity and rate of disappearance of the degradation products over time. The toxicity of partially degraded fractions should be determined, either from the literature or a bioassay. If the degradation products are toxic and are not degraded, then biodegradation would generally have to be considered unfeasible.

Laboratory experiments should compare degradation rates with leaching and volatilization rates. If these other rates are rapid in comparison with degradation, the soil properties may have to be modified for some distance along the pathways of migration to immobilize the biodegradable constituents.

It may also be necessary to add chemical reagents into contaminated soils to bring about detoxification or degradation reactions involving oxidation, reduction, or polymerization. The appropriate reagents, reaction rates, and the leachability and toxicity of reaction products will generally have to be determined by laboratory treatability studies.

## SECTION 3

## FIELD TESTS

Once the optimum conditions for biodegradation have been determined, an in-place method capable of achieving these conditions in the field has to be designed (Sims and Bass, 1984). Areas contaminated within 2 ft of the surface may require only simple tilling of the surface soils to introduce enough oxygen to enhance biodegradation. Depending upon laboratory results, the surface may also have to be inoculated with additional microorganisms, have the pH and nutrients adjusted, or the moisture content adjusted. Areas contaminated at greater depths will almost certainly require addition of oxygen and possibly microorganisms. It is conceivable that deeper compounds could be flushed out of the soil and reapplied in a controlled manner to the soil surface for biological treatment.

Soil attenuation might be used, if the level of contaminants is toxic to the organisms (Sims and Bass, 1984). In such a case, uncontaminated soil might be mixed with the contaminated soil to bring the concentrations to levels that could be successfully biodegraded. In practice, attenuation systems have been designed only for heavy metals; however, in principle, they should also apply to organic contaminants.

Since laboratory and pilot-scale testing do not simulate field conditions exactly, the effectiveness of the treatment process selected must be monitored after implementation. Soil core and soil pore water in the treatment zone and along migration pathways must be sampled and analyzed to determine whether the treatment process is functioning according to design. If the process is not as effective in the field as had been predicted by the laboratory or pilot studies, adjusting the operating conditions may improve the results.

The following are examples of pilot studies or field applications of in situ bioreclamation techniques.

3.1 Kelly Air Force Base, Texas. A small-scale soil and groundwater treatment system was used to circulate groundwater by pumping and gravity injection at a waste disposal site at Kelly Air Force Base, Texas (Wetzel, Davidson, Durst, and Sarno, 1986). This was the first field application of in situ biological treatment at a site contaminated with a complex mixture of both organic and inorganic wastes. The site was originally used as a disposal pit for chromium sludges and other electroplating wastes. Before it was closed in 1966, it had been used as a chemical evaporation pit for chlorinated solvents, cresols, chlorobenzenes, and waste oils. The site was chosen for demonstration of in situ biological treatment because the subsurface contained biodegradable organics and a highly adaptive and substantial population of microbes, the aquifer was not used as a source of drinking water, and the groundwater temperatures were near optimum for this treatment.

1. Background studies

These studies included site hydrologic and geologic characterization, identification of the contaminants, enumeration of the microbial population,

laboratory treatability studies, and an examination of the effects of nutrient and hydrogen peroxide addition on soil permeability.

Direct plate counts of organisms from subsurface samples ranged from 7.6 x $10^6$ to 1.7 x $10^8$ cells/g; viable cell counts ranged from less than 100 to 7 x $10^6$ cells/g. Similar yields of cells for seven different substrate media indicated the presence of highly adaptive bacteria.

Laboratory biodegradation studies investigated the potential of in situ degradation of the organic contaminants by indigenous bacteria (Science Applications International Corporation, 1985). Closed microcosms of 240 ml volumes contained soil and groundwater from the site. Aerobic microcosms were amended with nutrients and either hydrogen peroxide or oxygen. Anaerobic microcosms were also prepared in addition to aerobic and anaerobic sterilized controls. The microcosms were sampled at day 0, 25, 50, and 100 for volatile organic hydrocarbons and base/neutral and acid extractable organics. Significant degradation of aliphatic hydrocarbons (n-alkanes) and halogenated aromatics, such as chlorobenzene, occurred when oxygen and nutrients were supplied to the aerobic microcosms. Anaerobic microcosms showed degradation of chlorinated aliphatic compounds.

2. Treatment system configuration

This consisted of nine extraction wells and four injection wells arranged in a grid-like pattern within a 60-ft diameter circular area. The extraction wells pumped groundwater to a central surge tank, from which the water was released at a controlled rate to a distribution box. Nutrients and peroxide were pumped at controlled rates into the line between the surge tank and distribution box. An in-line baffle pipe provided mixing of the nutrients and peroxide with the groundwater. From the distribution box, the flow went to each of the four injection wells and was injected by gravity.

Groundwater was circulated for about two weeks prior to nutrient addition, which was followed by hydrogen peroxide addition two weeks later. The nutrient solution was Restore™ 375K Microbial Nutrient, supplied by FMC Aquifer Remediation Systems, Princeton, NJ. A 35 percent hydrogen peroxide solution, Restore™ 105 Microbial Nutrient (FMC Aquifer Remediation Systems), was added as the source of oxygen.

Hydrogen peroxide was initially added to maintain a concentration of 100 ppm in the groundwater, then increased by increments of 100 ppm every two weeks to acclimate the bacteria. The concentration was then maintained at 500 ppm.

3. Groundwater monitoring

A detailed monitoring schedule was implemented to routinely sample groundwater throughout the project from the wells and soil from the treatment zone to evaluate the effectiveness of the system. This was a critical component of the treatment system operation. Analysis of groundwater chloride, ammonium, and phosphate ion concentrations was important to determine whether nutrients were successfully transported through the subsurface and made available for microbial growth. It was found that nutrient transport was variable throughout the site due to differences in the hydraulic conductivity of the aquifer.

This monitoring indicated where modification had to be made to improve transport of nutrients to the areas that had been left untreated. Groundwater was, therefore, pumped from, and injected into, only those wells that had not shown nutrient transport. Frequent monitoring of the nutrients, chloride, carbon dioxide, dissolved oxygen, and pH help the system operator determine concentrations and volumes of treatment chemicals to be injected, as well as modifications required in pumping and injection patterns.

Carbon dioxide concentrations (an end product of microbial degradation and chemical oxidation of organics) were measured in the groundwater as a means of monitoring biological activity and system performance.

Field investigation indicated that the limiting factors for successful site remediation were the heterogeneous nature of the subsurface and the low hydraulic conductivity of the aquifer. The stratified nature of the subsurface and chemical precipitation of calcium phosphate from the treated groundwater resulted in slow transport of nutrients to some areas. Modifications in the treatment system pumping and injection patterns, system design, well placement, and equipment selection can help promote degradation in the less permeable areas of a treatment site.

4. Problems

Problems with this treatment method included a variable soil permeability, which prevented uniform pumping, and injection rates and aquifer heterogeneity, which required that the system design, well placement, and equipment selection (such as pumps and flow meters) be flexible for adjustment in the pumping and injections. This will help avoid migration of untreated contaminants. Precipitation of calcium phosphate in the distribution box and injection wells required addition of nutrients in a batch mode. Physical means, such as manual brushing the well screen and air surging, pumping, and bailing, alleviated the problem temporarily.

3.2 Pilot-scale field experiments were conducted (Ahlert and Kosson, 1983). A pilot-scale field installation, consisting of six soil columns, 2 ft in diameter and 5 ft deep, were exposed to leachate applications under natural environmental conditions. Experiments were conducted using specimens of sandy loam and clay loam soils and were designed for complete effluent recovery, allowing material balances. Vacuum was applied at the base of each column, balancing capillary forces and simulating natural drainage and water retention. The variables included hydraulic, organic carbon, and nutrient loading rates; soil type; and environmental conditions. A mixed microbial population derived from the secondary sludge of a municipal sewage treatment facility was added to the soil to supplement the indigenous population. The microbial population was cultured in the soil system, with daily additions of a dextrose feed solution for 10 days. At that time, the dextrose concentration was reduced, and the leachate was included as a supplementary organic carbon source. The microbial seed propagated through the soil column and permeated the entire soil structure. Nutrients were added to maintain a C-N-P-K balance, based upon TOC as the factor limiting biodegradation. The field experiment was operated for 169 days in 1982. Influent flow rates averaged about 10 liters/sq meter/day. Overall organic carbon removal exceeded 90 percent. Reductions in excess of 95 percent were readily attainable. The sandy loam demonstrated greater capacity

for volumetric throughput than the clay loam and would be a better choice for large-scale field application.

3.3 The following example illustrates some of the problems that can be encountered when setting up a field test. A field demonstration in a very gravely clay loam was largely unsuccessful due partly to low permeability (3.9 x $10^{-5}$ to 3.3 x $10^{-3}$ cm/s), which made it difficult to inject nutrients and produce water (Wetzel, 1986). The extreme complexities of the site and contamination problems also contributed to the poor success of this field demonstration. Problems encountered in the field demonstration portion of the project included 1) possible mobilization of lead and antimony by the hydrogen peroxide treatment, although the levels of these metals in the groundwater did not increase, and 2) reductions in the permeability of the soil due to precipitation of the nutrients. No significant evidence of biodegradation was observed in the first two months of hydrogen peroxide addition, but the nutrients had not reached most wells by this time (Wetzel, Henry, Spooner, James, and Heyse, 1985). After 23 weeks, elevated carbon dioxide levels, a metabolic by-product, were noted in production wells where the nutrients had broken through (Wetzel, 1986). Reductions in the concentrations of total hydrocarbon and individual organic pollutants, such as chlorobenzene, were noted.

## SECTION 4

## CASE HISTORIES

The following case histories document the use of biodegradation in actual contaminant incidents, as performed and reported by companies specializing in bioremedial operations. The incidents are summarized here, and the reader is advised to refer to the original publications for more details.

4.1 The groundwater at Ott/Story site was contaminated with a variety of volatile organics, phenols, aromatics, and other compounds at levels up to hundreds of mg/l and has a TOC that ranges from 600 to 1500 mg/l (Shuckrow and Pajak, 1982). Activated sludge cultures could not be acclimated to the groundwater even with the addition of nutrients and trace elements and adjustment of the pH or the addition of 10,000 mg/l powdered activated carbon. A commercial microbial culture was also ineffective. Coupling aerobic activated sludge with granular activated carbon adsorption (GAC) was effective in removing 95 percent of the TOC when the GAC worked effectively, but as GAC removal efficiency declined, so did the overall treatment effectiveness. The levels of most of the organic priority pollutants were also removed to below the limit of detection (0.01 mg/l). Some of the removal in the activated sludge process was due to air stripping, but microbial activity accounted for a large portion. The combination of GAC and activated sludge did not work well for groundwater from a less contaminated well having a TOC of only 200 to 300 mg/l. The affiliated company was Michael Baker, Jr., Inc., Beaver, PA.

4.2 As a result of leakage from unlined earthen pits used for burning waste fuel during crash-crew training exercises, JP-5 jet fuel contaminated 100,000 sq ft of a shallow perched aquifer at the U.S. Marine Corps Air Station in Tustin, CA (Ehrlich, Schroeder, and Martin, 1985). The pits had been present for at least 15 yr. A 5-ft clay layer provided an impermeable barrier to vertical migration to the deeper main water-bearing aquifer. Bacterial counts were 100 to 1,000 times higher inside than outside the zone of contamination. Only a few species of specialized bacteria, presumably those able to assimilate JP-5, were preferentially selected for in the contaminated zone. Contamination was predominantly by low-permeability materials. Thus, conventional depression-pumping methods alone might have been ineffective. An alternative scheme involved coupling biochemical activity with depression pumping. Some of the microorganisms could produce hydrocarbon emulsifiers, which could promote dispersion of pockets of trapped hydrocarbons from the sediments (Gutnick and Rosenberg, 1977). If dispersion and desorption effects were intensified, the rate of contaminant removal by depression pumping would be accelerated. In laboratory studies, addition of both potassium phosphate and potassium nitrate appeared to enhance microbial growth and cause emulsification of the fuel/water mixture. Sulfate-reducing and methane-producing bacteria apparently used fatty acids that resulted from the biotransformation of aliphatic and aromatic hydrocarbons in the JP-5 fuel. This information was used to develop a remedial plan.

4.3 In 1979, a leaking tank car released a large quantity of acrylonitrile in a Midwestern railroad yard (Walton and Dobbs, 1980). A recovery system removed the higher concentrations, and air stripping was used to treat the groundwater.

When the concentration of acrylonitrile was low enough to permit microbial growth, the recovery system was reversed, and mutant bacteria were injected into the contaminated site. The mutant, adapted microbes were combined with biochemical accelerators to formulate a product specifically designed to degrade organic chemicals. After an incubation and adaptation period of three months, the bacteria degraded the acrylonitrile to the lower detectable limit of 200 ppb. This treatment required careful monitoring.

4.4 Fumes discovered in four residences were traced to a leaking underground storage tank at a neighborhood service station, with an estimated loss of 30,000 gal of gasoline over 75,000 $ft^2$ (Brown, Norris, and Brubaker, 1985). Five pumping wells recovered 18,500 gal, and bioreclamation was initiated to remediate the soil and groundwater. Nutrients were periodically injected and air was continuously sparged over 10 months through 14 wells, and the gasoline level in the soil was reduced to less than 50 ppm. It was found that air sparging was able to maintain dissolved oxygen levels of only 1 to 2 ppm in the spill area. However, addition of microbial nutrient (a specially formulated, hydrogen peroxide-based nutrient solution; FMC Aquifer Remediation Systems, Princeton, NJ) raised DO levels to over 15 ppm, thus, establishing the efficiency of hydrogen peroxide-based solutions for supplying increased oxygen levels and, thereby, enhancing the bioreclamation process.

4.5 A long-term, low-level loss of 700 to 1,000 gal of gasoline from a buried underground service station tank into a weather-fractured bedrock aquifer contaminated 12 domestic water wells and an area of 87,000 $ft^2$ (Brown, Norris, and Brubaker, 1985). Remediation used a combination of air stripping to control VOC's with in situ bioreclamation to degrade the gasoline in place. The process consisted of injection of nutrients through a gallery located at the original leak source with air sparging in six monitoring wells. In the first 20 months, organic levels in the groundwater were reduced by 50 to 85 percent, at which point degradation stopped from inadequate oxygen supply. Microbial nutrient (nutrients plus hydrogen peroxide) was used as the oxygen source in place of air sparging. Six months later, eight wells were free of any contamination, and the other four had 200 to 1,200 ppb hydrocarbons.

4.6 Fumes discovered in a laboratory building at an industrial facility were traced to two leaking tanks in an adjacent tank vault (Brown, Norris, and Brubaker, 1985). From 700 to 1,400 gal of mixed fuels/solvents (45 percent aromatics/55 percent alkanes) were confined to the tank vault. Free product recovery of about 700 gal was followed by enhanced bioreclamation and ended with carbon treatment to finish off the groundwater. Groundwater levels during bioreclamation dropped from 22 to 45 ppm to <550 ppb in 2 1/2 months. The carbon treatment now became a cost-effective means of polishing the groundwater system.

4.7 An accidental spill of 130,000 gal of organic chemicals entered a 15-ft-thick, shallow, unconfined aquifer and produced contaminant levels as high as 10,000 ppm (Ohneck and Gardner, 1982). A 50- to 60-ft-thick gray silty clay separated the permeable aquifer from the main aquifer, which was a major source of drinking water.

A treatment system employing clarification, granular activated carbon adsorption, air stripping, and reinjection was assembled. An investigation into stimulating microbial activity showed that the contaminants were

biodegradable at concentrations below 1,000 ppm. Both the indigenous microflora and a specific facultative hydrocarbon degrader were able to biodegrade the materials in a soil/water matrix rapidly when supplied with nutrients. A biodegradation program was initiated that inoculated the treated water with the specific hydrocarbon degraders, nutrients, and oxygen.

Microbial growth responded positively to the presence of additional nutrients. The biological treatment process accelerated the removal of the compounds, as shown by a series of soil borings during the treatment process, and reduced the levels of contaminants in the groundwater to less than 1 ppm. The specific hydrocarbon degraders did not increase degradation in laboratory tests beyond that of the native microbes and may not have significantly increased *in situ* biodegradation. No data were presented that showed that the number of the specific hydrocarbon degraders increase in the subsurface or that they were able to outcompete the indigenous microflora in degrading contaminants. The affiliated company was O.H. Materials, Findlay, OH.

4.8 A gasoline spill from a storage tank in Millville, NJ, was found when gasoline fumes were noticed in basements adjacent to the gasoline station (Raymond, Jamison, and Hudson, 1978). An undetermined amount of gasoline was lost to the shallow, unconsolidated, sandy aquifer of high porosity and permeability. An extensive clay bed lay beneath the aquifer. Institution of a physical recovery program resulted in the recovery of 7000 gal, but the gasoline recovery rates decreased dramatically after two months to the point where only a few gallons were being recovered daily. The state agency in charge of the cleanup directed the operation to continue recovery operations until no trace of liquid gasoline remained on the groundwater surface, in order that the Millville well field be protected.

Preliminary tests before the bioreclamation operation began showed the presence of from $10^2$ to $10^5$ gasoline-utilizing organisms/ml in the groundwater. A biostimulation program was begun. Nutrients, including ammonium sulfate, disodium phosphate, monosodium phosphate, sodium carbonate, calcium chloride-dihydrate, magnesium sulfate-heptahydrate, manganese sulfate-monohydrate, and ferrous sulfate-heptahydrate were added to the wells continuously or in batches. Air was introduced into the wells by a carborundum diffuser with an output of 10 scfm and by diffusers constructed from DuPont Viaflow tubing that generated 1 scfm. Producing wells were used to control groundwater flow.

The microbial population responded to the addition of nutrients and oxygen with a ten- to thousandfold increase in the numbers of gasoline-utilizing and total bacteria in the vicinity of the spill, with levels of hydrocarbon utilizers in excess of $10^6$/ml in several wells. The microbial response was an order of magnitude greater in the sand than the groundwater. Forty-one cultures were isolated from the soil and groundwater at this site, with 17 considered to be *Pseudomonas*, four *Flavobacterium*, eleven *Nocardia*, and nine unidentified. Several of the *Pseudomonas* species were fluorescent, in contrast to the Ambler bacteria, which did not include fluorescent pseudomonads. Many of the cultures were composed of very small cells.

The biostimulation program was not completely successful; residual gasoline was found at the last sampling period, but no free hydrocarbon was observed in any of the wells after the biostimulation program ended. The gasoline concentrations in cores taken from the aquifer did not seem to change

substantially during the biostimulation program. Initially, the level of phenol in the groundwater was a problem, but it decreased to acceptable levels after more aerobic conditions were achieved in the aquifer. Gasoline levels in the produced water were generally quite low; although following an interruption in the biostimulation program, gasoline was found in the produced water, which indicated that pockets of gasoline remained in the aquifer. One well, which did not receive nutrients and air, was still contaminated with gasoline. This demonstrates that the biostimulation program did have an effect on the degradation of the gasoline. After the area near this well was treated, no free gasoline was found in any of the wells, and operations ceased with state approval. Microbial growth and utilization of nutrients and dissolved oxygen backed up the conclusion that enhanced microbial degradation was responsible for the removal of the gasoline. Suntech, Inc., Marcus Hook, PA, was the affiliated company.

4.9 Gasoline fumes from a gas station spill in Parkside, PA, reached the Parkside Elementary School and forced its closure (Suntech Group, 1978). Physical recovery was able to remove much of the gasoline and to lower the water table so that fumes did not reach the school.

Nutrients and oxygen were supplied to the groundwater microorganisms for six months. Tests showed that no excess hydrocarbon remained, but no data were presented to judge this claim. The fume problem was alleviated. The Environmental Group of Suntech, Inc., Marcus Hook, PA, was the affiliated company.

4.10 Gasoline fumes were detected in the basements of two restaurants and led to their closure in LaGrange, Oregon (Minugh, Patry, Keech, and Leek, 1983). The source of contamination was traced back to a bulk plant storage tank, which until recently had been owned by Chevron. A field investigation determined the boundaries of the gasoline plume and the hydrogeologic features of the site. The shallow groundwater (about 13 ft) roughly paralleled the land surface and was overlain by a top layer of topsoil, gravel, and silt at the surface; a five-foot section of assorted gravel, medium to coarse sand with some clay and silt; and a third layer about 4 ft in thickness, which was composed of medium to large cobbles, gravel, and coarse sand. A number of stream channel deposits crossed the site and provided highly permeable pathways for groundwater flow.

Laboratory biostimulation studies showed that the following nutrients were needed to stimulate the microbial population to degrade the gasoline: ammonium chloride, sodium phosphates, iron, manganese, magnesium, and calcium. A system was designed to draw down the static water levels 7 to 10 ft, to recycle the produced water (thereby, recycling nutrients and eliminating the problem of disposing of the water), and to supply nutrients and dissolved oxygen to the microbes. Groundwater was cycled through the site by installing three recovery wells in highly contaminated areas and injecting produced water into trenches upgradient of the area of known contamination. Air was supplied through a 2-inch line at the bottom of the injector trench with porous stone diffusers every 40 ft.

The physical recovery system accounted for recovery of 3,266 gal of free hydrocarbon. Nutrients broke through the production wells after only a few days, but high dissolved oxygen levels did not reach the recovery wells for five months due to its removal by microorganisms. Bacterial levels increased

up to six million times the initial levels. The system was operated for a year to encompass the complete range of seasonal water fluctuations. After nine months of treatment, soils in the highly contaminated tank storage area still showed signs of gasoline contamination at levels of 500 to 100 ppm and the average concentrations of dissolved organic carbon (DOC) was 20 ppm. After the biostimulation program ended, gasoline odors and a cloudy sheen were detected in some of the pits. However, samples showed continued improvement. The DOC in the water had fallen to the point where 71 percent of the measurements fell below 5 ppm and 50 percent were under 2 ppm. Since the DOC levels before treatment began were not given, it is difficult to evaluate the efficiency of the cleanup. Free product was no longer present in the recovery or monitoring wells, and the vapor problems at the restaurants were mitigated. The cleanup effort was ended with the establishment of an appropriate groundwater monitoring program. The affiliated companies were the Environmental Emergency Services Company, Portland, OR; Chevron U.S.A., Concord, AS; and consultants; Suntech Group, Marcus Hook, PA.

4.11 A number of spills and losses during transfers had contaminated the train yard of the German Railroad System in Karlsruhe with petroleum products (Nagel, et al., 1982). The hydrocarbons reached the groundwater table and caused a nearby water well to be closed. Cyanide was also found in the groundwater. The aquifer was composed of coarse sands and gravel mixed with pebbles and had a permeability factor of $2.1 \times 10^{-3}$ m/s.

A system was constructed in which contaminated groundwater was pumped out, treated with ozone, and then reinfiltrated via five injection wells. This treatment system controlled groundwater flow, improved the biodegradability of the contaminants, and provided oxygen to increase the activity of the microorganisms in the aquifer. Approximately 1 g of $O_3$/g of DOC was added to the groundwater and allowed a 4-min contact time. The oxygen content increased to 9 mg/l with a residual 0.1 to 0.2 g ozone/$m^3$ in the treated water.

This treatment was effective in restoring the aquifer. Dissolved oxygen levels increased in the wells with the most rapid changes noted in the monitoring wells closest to the infiltration wells. Oxygen consumption in the heavily contaminated zone reached 40 kg/day, but began to decline as the DOC levels dropped. The DOC concentrations fell to about 1.5 g/$m^3$, and virtually no mineral oil hydrocarbons or cyanide was detected. Total bacterial counts increased about tenfold, but bacteria potentially harmful to humans did not increase. The aquifer was restored to the point where drinking quality water was produced. The destruction of the hydrocarbons by ozone may have been more important in the cleanup than microbial activity. Microbial activity within the aquifer may have been limited since ozone is a toxicant (it is used as a disinfectant in some water distribution systems) and levels of inorganic nutrients were not supplemented. The affiliated company was the Federal Ministry for Research and Development.

4.12 An acrylonitrile spill contaminated the soil and groundwater of a site in Indiana (Polybac Corporation, 1983). Treatment was by pumping groundwater from several wells to a biotreator seeded with mutant bacteria from Polybac and then injection into the groundwater table. The concentrations of acrylonitrile fell from 1000 to 1 ppm within three months. The report did not contain sufficient details to judge the importance of microbial activity in the removal of the acrylonitrile. The affiliated company was Polybac Corporation, Allentown, PA.

4.13 The groundwater beneath a rail yard in a midwest metropolitan area was contaminated by a leaking rail car that released about 7,000 gal of acrylonitrile (Walton and Dobbs, 1980). The aquifer contained a significant amount of silt and clay and, consequently, had a low permeability. The groundwater table was quite shallow--only about 5 ft. The groundwater was treated by air stripping the recovered groundwater and, after concentrations had been reduced enough to allow microbial growth, by adding mutant bacteria. The degradation of acrylonitrile occurred rapidly, as the levels fell from 1,000 ppm to the limits of detection within a month. No data were presented that conclusively linked the drop in the concentration of acrylonitrile to microbial activity by the mutant bacteria. The affiliated company was Polybac Corporation, Allentown, PA.

4.14 A mixture of phenol and its chlorinated derivatives was spilled and reached the groundwater in central Michigan (Walton and Dobbs, 1980). Activated carbon filters were used to reduce the high concentrations of pollutants to levels tolerable by microbes. Mutant bacteria were injected into the soil and a surface run-off containment pond to degrade the pollutants. Degradation of phenol was rapid, but slower for o-chlorophenol. After an incubation and adaptation period, phenol was completely degraded from 31 ppm to below the lower detectable limit of 0.01 ppm within 40 days. However, the chlorinated derivative, ortho-chlorophenol, was reduced only from 120 ppm to 30 ppm during that time. This effort required careful monitoring. Once again the absence of carefully controlled experiments prevented any firm conclusions on the role of the mutant bacteria, although the data suggested that there was a correlation between the removal of the contaminants and the introduction of microbes. The affiliated company was Polybac Corporation, Allentown, PA.

4.15 A transportation accident spilled more than 100,000 gal of various organic compounds, including ethylene glycol and propyl acetate, over a 250,000-$ft^2$ area (Quince and Gardner, 1982). Three of the compounds penetrated the soil rapidly and were not removed when the contaminated surface soils were excavated. The soil consisted of a thick silty clay that extended to a depth of 50 ft. The water table was quite shallow and most of the contaminants were confined within it at a depth of 6 to 10 ft.

A series of 200 recovery wells were installed and the groundwater pumped to a treatment system employing clarification, aeration, and granular activated carbon adsorption. Once levels of the contaminants fell below 200 ppm, a biostimulation program was begun using special bacteria, nutrients, and air, which were injected into the surface.

The system was able to reduce the concentrations of ethylene glycol from 1,200 mg/l and propyl acetate from 500 mg/l to less than 50 mg/l and the total concentrations of spilled compounds from 36,000 to less than 100 mg/l. After the treatment had reduced the concentration of the contaminants to levels acceptable to the regulatory agencies overseeing the treatment, a further monitoring program revealed that the contaminants were below detectable limits. The importance of the microbes in the removal of the contaminants cannot be judged, although the authors noted that the inoculation and biostimulation program reduced the time necessary to reach the contaminant levels that met the regulatory agencies' approval. The affiliated company was O.H. Materials, Findlay, OH.

4.16 Groundwater contamination that resulted from leaks, overfills, and spillage at an industrial facility at an unknown location included dichlorobenzene, methylene chloride, and trichloroethane at levels up to 2,500 mg/l (Quince and Gardner, 1982). The site geology consisted of a fractured limestone bedrock overlain by weathered glacial deposits. The primary treatment was air stripping. The treated water was inoculated with commercial hydrocarbon-degrading bacteria and nutrients injected into the subsurface. The levels of bacteria increased until optimum conditions were established in the reactor and then were injected into the soil. In 2 1/2 months, the levels of methylene chloride fell from 2,500 mg/l to less than 100 mg/l and dichlorobenzene fell from 800 mg/l to less than 50 mg/l in a monitoring well. The inoculated bacteria were expected to continue to degrade the contaminants beyond the 95 percent reduction reached before the treatment was terminated. The importance of microbial activity could not be determined from the data presented. The affiliated company was O.H. Materials, Findlay, OH.

4.17 Four railroad tank cars derailed near Leon, Kentucky, spilling 390,000 l of acrylonitrile (Wetsel, Davidson, Durst, and Sarno, 1986). About one-third of this was recovered, and much of the remainder was burned or lost to the soil or a nearby river. The area had soils of a Muskingreen-Montivalle-Ramsey association. O.H. Materials was hired as a consultant for the cleanup and installed 16 aeration ponds to treat the contaminated water. The treated water was then injected into the soil. When the concentrations of acrylonitrile fell below 3 ppb, the water was released to the nearby river. The treatment process reduced the levels of acrylonitrile to less than 2 ppb, but the importance of biological activity was not estimated. The affiliated company was Atlantic Research Corporation, Alexandria, VA.

4.18 A solvent/fuel mixture of aliphatic and aromatic hydrocarbons had contaminated the soil under a tank field (Brown, Longfield, Norris, and Wolfe, 1985). After the free solvent was pumped off, the soil contained 300 to 800 gal hydrocarbon in 800 cubic yards of soil. This soil was treated by recirculating nutrient and oxygen-enriched water (at 25 gpm) through the contaminated area. Two pairs of interceptor and injection wells were used. Nitrogen and phosphorous were added in the form of ammonium chloride and sodium phosphate. Oxygen was supplied by adding hydrogen peroxide to the water. This treatment lowered groundwater hydrocarbon from 23 to 0.5 ppm in 2 1/2 months, indicating that most of the hydrocarbon on the soil had been degraded. In this case, carbon was used to finish the groundwater cleanup.

4.19 Some 18,000 gal of gasoline were removed from soil contaminated by a leaking UST (Brown, Longfield, Norris, and Wolfe, 1985). Groundwater contamination was reduced from 30 to 40 ppm to less than 1 ppm. Soil gasoline content was reduced from 2,000 to 3,000 ppm to less than 50 ppm. This required pumping groundwater at 30 gpm for 10 months. Air sparging resulted in only 1 to 2 ppm dissolved oxygen (DO), while peroxide was able to raise DO to 15 ppm. This enhanced the biological activity in the soil.

4.20 An orthochlorophenol spill in Missouri contaminated soil and a pond (Anonymous, 1981; Anonymous, 1982). It was treated by Polybac Corporation. A spray/injection leachate system was built using the pond as a treatment reactor. The pond was seeded with Polybac's products and the concentration of orthochlorophenol fell from 15,000 ppm to less than 1 ppm within nine months.

4.21 The Amoco Cadiz oil spillage released over 2 x $10^5$ tons of oil; more than 320 km of coastline was oiled (Atlas and Bronner, 1980). Intertidal microbial communities along the Brittany coast impacted by the oil contained elevated proportions of hydrocarbon utilizers for more than one year following the spillage. The average biomass of intertidal hydrocarbon utilizers was estimated at 50 g/hectare. Rates of utilization of n-alkanes were greater than those for branched alkanes, which were greater than those for two-to four-ring aromatics. Degradation of polynuclear aromatics appeared to be largely due to cometabolism.

4.22 The Ixtoc I well blowout resulted in the largest oil pollution incident to date (Roubal and Atlas, 1980). Approximately 3 to 15 miles from the wellhead, a water-in-oil emulsion (mousse) formed. Populations of hydrocarbon-utilizing microorganisms in mousse-associated surface waters ranged from $10^3$ to $10^4$ hydrocarbon utilizers/ml; in control samples free of mousse, concentrations of hydrocarbon utilizers were lower than $10^{-1}$/ml. This implies microbial oxidation in the formation of mousse in addition to physico-chemical processes. The mousse was stable. Long-term, slow mineralization of mousse was observed but rates of mousse hydrocarbon mineralization were significantly lower than normally found for nonemulsified petroleum hydrocarbons. Populations in Gulf of Mexico water were capable of mineralizing 10 to 20 percent of n-alkanes within two weeks in nonemulsified oil but less than 10 percent of the mousse was mineralized within 10 weeks.

4.23 In March 1982, a train tank car containing formaldehyde was vandalized in the middle of a community (Sikes, 1984). The valve on the bottom of the tank car was opened, and the 20,000 gal, 40 percent solution of formaldehyde was discharged along the siding causing the solution to run into an adjacent ditch and river. Most of the formaldehyde was physically removed and hauled to a hazardous waste landfill. Polybac Corporation biologically decontaminated the soil and rail ballast without having to disrupt train operations. An on-site biological treatment system and surface spray system was designed to achieve the end goal of 1 ppm leachate in the ballast and soil. The biological system and surface spray system were installed on-site in a day. The concentration of leachate from the soil and ballast material was more than 700 ppm formaldehyde. Within 24 days of operation, the concentration had dropped to 1 ppm.

The hydrogen peroxide oxidation was successful in lowering the formaldehyde concentration to levels where biological oxidation could be implemented. A novel approach that converted the rail ballast into a biological trickling filter was used. The process operated in the following manner. Leachate from the ballast material drained into the adjacent ditch, which was dammed at either end to act as a sump. A sump pump was placed in the ditch to pump the high concentration formaldehyde into the biological treatment unit. The biological treatment unit was inoculated with HYDROBAC$^{tm}$, appropriate nutrients were added, the pH was adjusted, and the treatment unit was aerated. The discharge from the biological treatment unit was pumped by means of a spray system over the contaminated ballast and soil increasing the leaching of the formaldehyde. Biological activity was initiated in the ballast zmaterial and soil by the biomass being sprayed on it.

Costs for the biological remedial cleanup of the soil were estimated at $50,000 and delay in operation at $80,000/hr. Landfilling of removed ballast

would have added $75,000 for a total estimated physical decontamination cost of $445,000.

4.24 At a high-octane gasoline spill in Whitemarsh Township, PA, stimulation of an indigenous microflora population was successfully utilized to degrade the organics contaminating a dolomite aquifer (Chapin, 1981). Added nutrients and air stimulated biodegradation of the gasoline residuals. Biodegradation required about two years. Approximately 95 percent of the residual material was degraded.

4.25 In 1979, 1980, and 1985, leaks were detected in two fuel farms at a United States Coast Guard (USCG) air station at Traverse City, Michigan (Brown, Loper, and McGarvey, 1986; Wilson, Bledsoe, Armstrong, and Sammons, 1986). The farm for aviation gasoline (115/145 AVGAS) was found to have been leaking since 1969. Fuel components, such as benzene (2,500 ppb), toluene (70,000 ppb), and xylene(s) (1,000 ppb) (BTX) had dissolved in high concentrations in the groundwater. Total alkylbenzene concentrations near the center of the plume were approximately 30 mg/l; there were high concentrations of methane; and there was no detectable oxygen. The heart of the plume was surrounded by an anaerobic zone with greatly reduced concentration of alkylbenzenes and no oxygen. Laboratory studies confirmed anaerobic transformations of the alkylbenzenes by one order of magnitude by the end of eight weeks of incubation. The anaerobic zone was surrounded by an aerobic zone with detectable oxygen and even greater reductions in alkylbenzene, reflecting rapid aerobic transformations of the material, by two orders of magnitude by the end of two weeks incubation. Around this area was a renovated or pristine zone, with high concentrations of oxygen and no alkylbenzenes.

The Traverse Group, Inc., an Ann Arbor-based, multidisciplinary consulting firm, handled the cleanup. Pilot-scale air stripping and carbon adsorption systems were set up, and an interdiction field installed.

The other leaks were detected at the fuel farm for jet fuel (JP-4). Three of four fiberglass underground storage tanks were leaking, and there were numerous leaks in the piping systems. The fuel farm had to be abandoned. To date, 24 to 31 inches of product is still floating on the water table in this area. Benzene, toluene, and lighter hydrocarbons are the major components. The spread of contamination down-gradient is controlled by a product recovery system and a four-well purge field. The plume is approximately 4,300 ft in length and from 180 to 400 ft in width; its vertical dimension ranges from 25 to 80 ft.

Much of the organic material is retained in a 6- to 12-inch-thick layer in the capillary zone immediately above the water table, which is possibly slowly leaking organics into the groundwater. Usually, contaminants, such as benzene and toluene, are mainly found in the upper portion of the aquifer. However, this spill had been in the ground so long that it had mixed down-gradient throughout the vertical cross section of the aquifer.

A serendipitous activation of the naturally occurring soil microbial community resulted in a rapid and dramatic reduction in the contamination in some areas of the plume. As toluene levels fell from 10,329 ppb to less than 10 ppb in about 100 days, benzene levels rose, possibly due to demethoxylation of the toluene to benzene. Then levels of both compounds fell.

4.26 During the late 1960s, an excavated sand pit was fill with household refuse, demolition materials, chemical sludges, and hazardous liquid chemicals (Stover and Kincannon, 1983). In the late 1970s all further disposal of hazardous wastes was prohibited, and cleanup activities began. Batch-activated sludge treatability studies were conducted on the lime-treated groundwater using seed sludge from several operating systems that were dealing with many of the organics found in the groundwater. After a three-week acclimation and stabilization period, the sludge was used in the batch tests. The biological treatment effectively removed TOC, total phenols, and the synthetic organics of concern.

4.27 A large gasoline spill (about 250,000 gal) occurred in Glendale, CA, contaminating an irrigation well (Williams and Wilder, 1971). Several bacterial species of Pseudomonas and Arthrobacter could utilize the gasoline when supplied with trace nutrients and adequate dissolved oxygen (McKee, Laverty, and Hertel, 1972). Bacterial degradation of trapped gasoline was more rapid in the zone of aeration above the water table than in the water-saturated zone. The levels of gasoline-utilizing bacteria in contaminated wells were 50,000/ml or higher and gradually fell to the background level of 200/ml as the gasoline disappeared. It was suggested that under favorable conditions, these bacteria might be useful for a final cleanup of underground spills.

4.28 In situ biological treatment was used to clean up oil-contaminated soil at a fuel transfer depot at a site in a middle Atlantic state, with about 20 underground tanks (Thibault and Elliott, 1979). Poor housekeeping practices during loading operations over the years had led to contamination of the soil surface over a one-acre site. The soil was first tilled vigorously with conventional farm equipment to ensure an adequate supply of oxygen and good microorganisms-hydrocarbon contact. The proper nutrient balance was ensured through the application of 500 pounds of a commercially available nutrient source (POLYBAC® N Biodegradable Nutrients). Then, an aqueous solution containing 80 pounds of a commercially available biodegradable emulsifier (POLYBAC® E Biodegradable Emulsifier) was sprayed over the contaminated soil. Finally, the area was inoculated with 50 pounds of a dry, mutant bacterial hydrocarbon degrader (PETROBAC Mutant Bacterial Hydrocarbon Degrader) following a 2-hr rehydration period in warm water. The area was tilled regularly over the summer months and the soil was kept moist naturally by the rain.

4.29 In August 1975, a small creek that discharges into Allendale Brook, a New Jersey state stream, was found to be contaminated with an estimated 33,000 gal of the following substances (Jhaveri and Mazzacca, 1982; Jhaveri and Mazzacca, 1985):

| | | |
|---|---|---|
| Methylene chloride | - | 181,500 lbs |
| n-Butyl alcohol | - | 66,825 lbs |
| Dimethyl aniline | - | 26,300 lbs |
| Acetone | - | 10,890 lbs |

A leak in an underground process line from Biocraft Laboratories, Inc., a semisynthetic penicillin manufacturing plant, had infiltrated a storm sewer line that discharged into the stream.

The contamination was confined to an 8- to 15-ft layer of glacial till, with a poorly sorted mixture of boulders, cobbles, pebbles, sand, silt, and clay. Permeability was highly variable throughout the till layer, varying from 1 to 35 gpd/ft$^3$, and the ground water migrated at an average rate of 0.4 ft/day. About 40 feet of semiconsolidated silt and fine sand underlay the till layer, and acted as an aquitard to separate the shallow aquifer from a shale layer, which served as the drinking water supply for the region. Groundwater movement was fairly rapid at 0.4 ft/d in the shallow water table. The permeability of the glacial till varied from 1 to 35 gpd/ft$^2$. Visual inspection suggested very low permeability. Brunswick Shale underlay the semiconsolidated clay for several hundred feet.

Ten monitoring wells were installed. Initially, 10,000 gal of contaminated groundwater were collected per month and sent to an approved disposal facility. Soil column studies indicated that flushing the contaminants from the soil and collecting contaminated groundwater for off-site disposal would take 30 to 50 years. Removal and disposal of contaminated groundwater was improved from a rate of from 2,500 to 10,000 gal/month after installation of 12-inch pumping wells. The average cost of disposal was $0.35/gal.

In August 1981, Groundwater Decontamination Systems, a subsidiary of Biocraft, initiated an on-site biostimulation-biodegradation system to accelerate decontamination of the site. It was estimated that the system would require five years to complete the cleanup using biodegradation (Amdurer, Fellman, and Abdelhamid, 1985).

1. Initial study

Contaminated groundwater was inoculated with various soil samples to find organisms that would biodegrade methylene chloride. Soil from the contaminated site was the most promising. A shake flask study used contaminated water as the sole carbon source. The following medium was used:

| | |
|---|---|
| $NH_4NO_3$ | 100 mg |
| $Na_2HPO_4.7H_2O$ | 40 mg |
| $KH_2PO_4$ | 100 mg |
| $MgSO_4.7H_2O$ | 20 mg |
| $Na_2CO_3$ | 100 mg |
| $CaCl_2$ | 1 mg |
| $MnSO_4$ | 2 mg |
| $FeSO_4.7H_2O$ | 0.5 mg |
| per liter | |

However, an increase in methylene chloride concentration required an increase in dibasic phosphate to buffer the acid formed due to the oxidation of methylene chloride. A similar anaerobic study was not successful.

Microorganisms isolated from the activating tank consisted of: Pseudomonas (40 percent), Agrobacterium (40 percent), and Arthrobacter (20 percent). These are naturally occurring soil organisms, known to use a wide variety of organics as a carbon source.

2. Field feasibility study

A pilot study in one of the monitoring wells demonstrated the feasibility of a biostimulation program. Aeration of the groundwater (temperature 12 to 14°C) in a monitoring well with a small sparger and the subsequent addition of nutrients resulted in an increase of bacteria from $1.8 \times 10^3$/ml to $1.6 \times 10^6$/ml in a 7-day period.

3. Batch process study

This study was carried out in a Chemap 14-liter glass fermentor containing 10 liter of contaminated groundwater. Mineral salts were added, and air was sparged through a fritted glass tube at 0.6 liters/vol/min. The effluent air was passed through activated carbon, and biodegradation was followed by COD and total bacterial counts. There was a large decrease in COD correlating with a large increase in total counts in eight to nine days. This is evidently an acclimation period for the organisms. The COD values had leveled off by the twelfth day, indicating completion of the biodegradation. The lag phase could be reduced to two days by using an acclimated inoculum. There was no significant difference between 20 and 30°C.

4. Continuous biodegradation process

Two Chemap 14-liter fermenters were set up in series. One was a biodegradation (activating) unit containing 12 liters of contaminated groundwater. The other was a settling unit. A 5-gal reservoir was filled with contaminated groundwater, and essential minerals were added to the waters. A multichannel peristaltic pump was used to pump contaminated water into the activating unit at the rate of 700 ml/hr. The second line pumped treated water from the activating unit into the settling unit, and a third line pumped supernatant from the settling unit to an effluent reservoir. This process had a 17-hr retention time. Methylene chloride was reduced by about 90 percent after four days. However, the amount of acetone increased, since isopropyl alcohol is transformed into acetone under aerobic conditions. Dimethyl aniline was also reduced by 90 percent.

5. Pilot-plant study

This was carried out in 55-gal drums on their sides in series, with one as the activating unit and the other as the settling unit. The first received 45 gal contaminated groundwater and was inoculated with 10 percent inoculum from the laboratory experiments. Air was sparged through porous alumina air diffusers at a rate of 0.4 liters/liter/min. Contaminated groundwater was fed into the activating unit at the rate of 12 liters/hr. The temperature was 20°C, and the retention time was 16 to 17 hr. The effluent was pumped to the settling unit where the supernatant was pumped out and the settled biomass returned to the activating unit. Pilot plant studies ran for about 17 days and provided basic information for the design of the biological treatment plant.

6. Site decontamination system

The biostimulation-decontamination system consisted of:

1. A groundwater collection system down-gradient of the contaminated plume

2. A four-tank dual aerobic biological treatment system

3. A series of *in situ* aeration wells to maintain aerobic conditions along the path of groundwater flow

4. Effluent injection trenches up-gradient of the contaminated plume

5. Control room

A hydrogeological survey was performed so that a system to confine the contaminated groundwater on-site and decontaminate it could be designed. The contaminated plume was collected downgradient for treatment in a surface biological treatment system. The collection system consisted of a trench, about 80 ft long, 4 ft wide, and 10 ft deep. Two slotted collection pipes on a gravel bed in the trench were connected to a central collection pumping well. The trench was backfilled with washed stone and covered with 15-mil plastic sheet and backfilled with earth to grade. The pumping wells were installed by digging an open hole about 4 ft by 6 ft by 10 ft deep. A 12-inch PVC slotted casing was installed and the trench backfilled with washed stone. A sump pump with a 10-gpm capacity was installed in the wells. Two 6,000-gal tank wagons were used for the biodegradation, and two 5,000-gal wagons for the settling tanks. Air was supplied to the air diffusers in the activating tank from an air blower. A total of 20 scfm was supplied to each activating tank containing 5,000 gal of contaminated groundwater. Following a residence time of 16 to 18 hr in the activating tank, the effluent was pumped to the two settling tanks. About 200 gal of the activated sludge slurry was recycled to the activating tanks daily. The effluent from the settling tanks, which was enriched with organisms, nutrients, and some oxygen, was supplied with additional oxygen and recirculated *via* the reinjection trenches. The following compounds were used in the nutrient solution: 500 mg/l $NH_3Cl_2$, 270 mg/l $KH_2PO_4$, 410 mg/l $K_2HPO_4$, 18 mg/l $MgSO_4$, 9 mg/l $Na_2CO_3$, 1.8 mg/l $MnSO_4$, 0.45 mg/l $FeSO_4$, and 0.9 mg/l $CaCO_3$. The system treated 10,000 gpd with a 0.5-gpm feed stock flow and retention time in the activating tank of 17.5 hr. The novelty in the process was the extended biological surface treatment--16 hr--under optimum conditions.

A series of nine air injection wells were installed in the subsurface along the major pathway of the groundwater flow and spaced about 30 ft apart. Air was injected into each well at a pressure of 4 psig. The radius of influence of each air well was greater than 15 ft. Average groundwater flow through the aerated zones was 0.4 ft/day. Groundwater temperatures ranged from 10 to 12°C, providing adequate conditions for *in situ* biodegradation, as the nutrient-rich water passed through the aerated zone. A small control room contained the equipment for monitoring and adjusting rates, as required.

7. Biological treatment plant

The initial treatment was a batch operation to grow the organisms. Each activating tank wagon contained 4,000 gal of contaminated water and was inoculated with 50 gal of inoculum from the pilot plant. Mineral salts were added, air was sparged at the rate of 50 scfm, and the temperature maintained at 20 to 25°C.

After a month, the system became continuous. The influent to the activating tank was increased to match the recharge rate of the pumping wells. The air was reduced and nutrient concentration adjusted. Air was sparged at a rate of 20 scfm and the temperature maintained at 20 to 25°C. The effluent air was passed through an activated carbon filter, and the treated water pumped to the settling tanks at a rate to maintain a balanced system. Sludge production was minimal, since some of the sludge was recycled from the settling tanks to the activating tanks and some was allowed to pass with the supernatant to the recharge trenches to inoculate the soil with acclimated microorganisms.

8. Project and operating costs

The total cost for the biostimulation project is tabulated below:

| | | |
|---|---|---|
| 1. | Research and development | $453,399.00 |
| 2. | Hydrogeological design and construction | 184,243.00 |
| 3. | Process plant design and construction | 221,207.00 |
| | | $858,849.00 |

The total dollars spent include all aspects of the three individual costs. Elimination of the cost of the learning curve projects the cost at the Biocraft site to be approximately $300,000.00.

Based upon treating 13,500 gpd, the total operating cost, including utilities, nutrient cost, labor, maintenance, and overhead, was $0.0165/gal. The total cost, including amortization based on projected cost, was $0.0358/gal over a three-year period.

After three years, capital costs were $926K, and the Operating and Maintenance costs were $226/day (Environmental Protection Agency, 1984b). The total cost with biodegradation was estimated to be one-fourth that via the initial remedial measure (pumping and off-site disposal) (Amdurer, Fellman, and Abdelhamid, 1985). The preliminary research and pilot studies accounted for about $450,000, or one-half of the total capital cost. A significant investment is necessary in site-specific research. Treatability studies and research are a fundamental part of in situ remediations.

After five years, total charges for the project had come to about $40/cu yd of fully decontaminated soil (Rich, Bluestone, and Cannon, 1986). This compares favorably with other methods of soil treatment, including bioreclamation. Capital costs were $405,000, and $520,000 was spent on research and development. Costs for treating 13,600 gal of groundwater daily were 1.65 cents/gal, in contrast with 35 cents/gal that Biocraft had previously paid to have the groundwater shipped off-site for disposal. The affiliated company was Ground Water Decontamination Systems, Waldwick, NJ.

This system was patented by Groundwater Decontamination System. After three years of operation, the size of the original contaminated plume was reduced by 90 percent, based on water quality data and continuous core samples (Alexander, 1985). A core sample analyzed from 0 to 12 ft showed no contamination at the detection limit of 0.8 ppm. Biodegradation was demonstrated by a 10-fold increase of carbon dioxide production in various monitoring wells in the contaminated area, as compared with background levels.

Carbon dioxide evolution was found to return to background levels after biodegradation.

After five years, 90 percent of the contaminants in the groundwater and soil had been cleaned up (Rich, Bluestone, and Cannon, 1986). The remainder was difficult to remove, mainly because of the soil's low permeability. Carbon dioxide levels show that the biological action continues.

The above ground system was able to remove about 95 percent of the organics, but some of this may have been due to air stripping. The contaminant levels in one of the pumping wells went from 91 ppm methylene chloride and 54 ppm acetone to less that 1 ppm within a year of treatment. The COD of the groundwater was also significantly reduced. The remaining contaminated area still contained detectable levels of the contaminants, but there was evidence they were rapidly biodegrading.

4.30 In July 1971, the Ambler Borough Water Department, Ambler, PA, notified Sun Pipe Line Company that gasoline was in the water at a pump station that provided 1 million gal of water/day to the surrounding community (Raymond, Jamison, and Hudson, 1976). A leaking pipeline had released 3,186 barrels of high-octane gasoline into the environment. Forty-six wells were drilled in the affected area and free product recovered. This became nonproductive in less than a year. An estimated 1,000 barrels remained, which might have taken 100 yr for the water to recover and reach a potable level. Biological stimulation of the indigenous hydrocarbon-utilizing microbial flora was carried out. When nitrogen, phosphate, and oxygen (via forced aeration) were supplied, gasoline-utilizing bacteria multiplied and gasoline removal accelerated (Blakebrough, 1978).

It was decided that the feasibility of carrying out large-scale stimulation of the indigenous flora to degrade the gasoline in the groundwater should be determined. From a laboratory and pilot field studies, it was demonstrated that natural flora of gasoline-utilizing organisms were present at levels of $10^3$/ml (Jamison, Raymond, and Hudson, 1975). This population could be increased a thousand-fold by supplementing the groundwater with air, inorganic nitrogen, and phosphate salts. Eighteen months after the pipeline break, a full-scale program for stimulating hydrocarbon-utilizing bacteria in the groundwater was initiated.

Field Test

A small field test of one of the wells was conducted. Air was first diffused into the well, to within 1.5 m of the bottom. Then a 30 percent concentrate mineral solution was added at a rate of 75 l/hr to the injection wells from a 2,200-gal tank truck. It consisted of ammonium sulfate (15 kg), disodium phosphate (8.2 kg), and monosodium phosphate (6.8 kg), dissolved in 150 l of water. The oxygen level decreased after one day and the bacterial count increased, probably as a result of the increased utilization of gasoline. No change was noted until sufficient oxygen had been supplied. It was concluded that aeration combined with nutrient addition was essential to stimulate the microbial flora, which would accelerate the removal of the gasoline remaining in the reservoir.

It should be possible to estimate the amount of nutrient and oxygen required based upon cell composition and conversion of hydrocarbons to cells. For example: assuming a cell composition of 7 percent nitrogen, 3.5 percent phosphorus, a requirement of 544 g oxygen/453.6 g cells synthesized, and a conversion rate of gasoline to the cells of 80 percent, the amount of N, P, and $O_2$ required would be on the order of $6.6 \times 10^3$ kg, $3.3 \times 10^3$ kg, and $1.1 \times 10^5$ kg, respectively. Addition of these quantities of nutrients to the well should accomplish removal of the gasoline in a matter of months rather than the years that physical removal would have required.

Full-scale Program

Aeration. Air was sparged into 48 wells using 10 diffusers attached to paint sprayer-type compressors capable of delivering 2.5 cu ft/min (0.07 $m^3$ of air/min).

Sampling. Sterilized 6-oz glass bottles were secured near a weight on the end of a sash cord. When a bottle was lowered into place, a cork in the bottle was removed by a sharp jerk permitting filling of the bottle, and the sample was used for chemical and microbiological analyses. DO concentrations in individual wells were determined infrequently throughout the year, since it was necessary to sample when mineral concentrations were less than 2,000 ppm. Otherwise, "salting out" of oxygen would give misleading values.

Analytical Methods. Chemical analyses were carried out using Standard Methods (APHA 1955; 862). Ammonium sulfate, disodium phosphate, and monosodium phosphate were added to the water wells as food-grade chemicals. Dissolved oxygen (DO), nitrate, nitrite, and phosphate were determined using prepackaged chemicals (Hach Chemical Company, Ames, Iowa 50010). Ammonia was determined by nesslerization, described in "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association, 1971). There was a fairly good correlation between calculated yields of gasoline utilization based either on nitrogen or phosphorus uptake in the reservoir. These were based on nitrogen and phosphorus concentrations in cells and percent conversion of gasoline to cells.

It was determined that additional nutrients would have to be added to obtain maximum utilization of the gasoline. $NH_4^-$ or $_3^-$ could serve equally well as a nitrogen source, and magnesium, calcium, and iron were not required in this situation.

Bacterial Studies. Total counts on nutrient agar (NA) were determined by the standard pour-plate method, reading serial dilutions at seven days. The gasoline-utilizing organisms were enumerated by the dilution spread-plate technique by spreading a drop from serial dilutions on a mineral salts (MM) agar plate and counting at 14 days. The gasoline-utilizing bacteria were isolated from MM medium with Sunoco 260 as the sole source of carbon. The plates were incubated at 13°C in a vacuum desiccator under gasoline vapors from a 125-ml filtering flask, containing gasoline, attached to the desiccator.

Very high biological activity was present in wells in the vicinity of the immediate spill area. Addition of nutrients to the groundwater had varying degrees of stimulation in individual wells, as measured by the plate count method, probably as a result of availability of gasoline for growth. Foaming

was encountered in several wells with high bacterial numbers and was assumed to indicate vigorous bacterial growth. Final counts were very low, probably reflecting gasoline and nutrient availability.

Over 18 months, 32 cultures that were considered to be the predominant gasoline-utilizing flora were isolated from the groundwater. Seventeen were gram negative and 15, gram positive. Eight of the gram negative may have been pseudomonads; four were Acinetobacter; and one was Flavobacterium devorans. Ten of the gram positive appeared to be actinomycetes of the genus Nocardia. Two were of the genus Micrococcus. It was found that the naturally occurring bacterial flora could assimilate specific hydrocarbons (toluene and n-paraffins), as well as the gasoline itself. Combined, these organisms could degrade gasoline, but no single isolate could utilize all the components of gasoline. Nocardia were probably responsible for the major paraffinic hydrocarbon component degradation, and Pseudomonas for degradation of the aromatics. Either cooxidation of hydrocarbons plays a very large role during stimulation or else a significant number of species that could grow on branched paraffins and naphthenes was not isolated. Die-off of gasoline-utilizing bacteria did not begin until a year after initiation of the stimulation program.

Nutrient Injection. Each well was injected with 30 gal of a 30 percent nutrient concentrate. Changes in phosphate concentration in water samples permitted calculation of the reservoir size around each well and the magnitude of the flow that could be expected away from each injection site, to optimize nutrient concentrations in the high gasoline-contaminated area.

Problems. The most difficult problem encountered in optimizing growth in the reservoir was the distribution of nutrients. The lack of homogeneity of the dolomite formation and the shifting clay made the injection and distribution of inorganics difficult. The bacteria were subjected to wide variations in osmotic pressure as the result of the 30 percent "concentrate" injections, undoubtedly stopping growth. Due to shifting water patterns and the problems of handling dilute solutions on a continuous basis, it was not feasible to provide more optimum concentrations.

Six months after the addition of nutrients had stopped and 17 months after initial microbial stimulation, bacterial counts were very low, and samples had no detectable gasoline. The low counts probably reflected the reduction in gasoline and nutrients. Continuous monitoring of gasoline by an ultraviolet detector showed the water to be free of gasoline (<500 ppb) 21 months after onset of the biodegradation. Indirect evidence (nitrogen and phosphate retention in the reservoir, high bacterial population in wells) suggested that more than 1,000 barrels of gasoline were removed in the process, and the data provided conclusive evidence that addition of nutrients accelerated bacterial growth and biodegradation of the gasoline. A map of the bacterial counts following the gasoline spill resembled the contours of gasoline contamination (Raymond, Jamison, and Hudson, 1975).

# APPENDIX F

# BIOTECHNOLOGIES IN REMEDIATION

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## LIST OF TABLES



## LIST OF ILLUSTRATIONS

# SECTION 1

# BIOLOGICAL PROCESSES FOR REMOVAL OF ORGANIC CONTAMINANTS FROM GROUNDWATER

## 1.1 ACTIVATED CARBON SYSTEMS

Carbon adsorption is effective in removing low levels of some organic contamination (Brubaker and O'Neill, 1982). Activated carbon systems can be batch, column, or fluidized-bed reactors (Lee and Ward, 1986). Batch systems typically use powdered carbon for application, such as protecting biological treatment systems or when low capital costs and ease of operation are desirable. Column systems can be single or parallel adsorbers, which are useful in high volume flows or where a pressure drop is expected, ad have moderate adsorbent costs. In contrast, adsorbers in series produce a gradual breakthrough curve, can be used continuously, can give lower effluent concentrations, but have higher adsorbent expenses. Expanded upflow systems have been employed for high flows containing considerable quantities of suspended solids, whereas moving bed reactors provide efficient carbon use for wastewaters with low amounts of suspended solids and minimal biological activity is expected.

Carbon adsorption systems are sensitive to the composition of the influent, to flow variations, to fine precipitates, to oil and grease, and to suspended solids in the influent water (Lee and Ward, 1986). They may be clogged by biological growth, although this growth may provide additional treatment by destroying organics. Activated carbon systems have a finite loading capacity. They may be regenerated by a high temperature burn, which is expensive, or by treatment with steam or a solvent. The spent carbon may also be placed in secure landfills or other sites that isolate the carbon and do not allow any desorbed organics to contaminate other environments.

The effectiveness of carbon adsorption is controlled by the tendency of the contaminated species to fit into the micropores on the surface of the carbon (Brubaker and O'Neill, 1982). It is most often used with aromatics (including chlorinated aromatics, phenols, and PAHs), fuels, chlorinated solvents, and high molecular weight amines, ketones, and surfactants. Because compounds that are either much larger or much smaller than these materials (on a molecular level) do not fit into the pores, they are not generally good candidates for carbon treatment. Like biodegradation, a mixture of materials might not respond like the sum of its individual parts. There are many compounds that inhibit the adsorption of other contaminants to a carbon surface. In addition, those materials that adsorb most effectively to carbon also adsorb effectively to the soil and are, thus, difficult to transport into the water in the first place.

### 1.1.1 Biological Activated Carbon Systems

If organisms are immobilized on a granular activated carbon filter, enhanced biodegradation of industrial aromatic effluents can be promoted (Roberts, Koff, and Karr, 1988). Refractory aromatic compounds, such as indole, quinone, and methylquinone, have been successfully degraded with this method in concentrations over 300 mg/l.

This system removes biodegradable and biorefractory materials within the same treatment unit (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Tsezos and Benedek, 1980). It is a variation of the carbon adsorption process and can be in either of two forms:

1. Addition of powdered, activated carbon to aeration basins of biological systems

2. Biological activated granular carbon in a fluidized-bed reactor

The first form is relatively simple, since only the carbon feed system is required. Part of the carbon is recycled from clarifiers back to the aeration basins, while other spent carbon is removed from the system together with waste-activated sludge, which can be either disposed of or regenerated in a wet-air oxidation unit.

Benefits of this method are a high biological oxygen demand (BOD) and chemical oxygen demand (COD) reduction, despite hydraulic and organic overloading; an aid to solids settling in the clarifiers; a high degree of nitrification due to extended sludge age; substantial reduction in phosphorus; adsorption of toxic compounds and coloring materials, such as dyes; adsorption of detergents; and reduction of foams.

In the case of a fluidized-bed biological reactor, the granular activated carbon bed is expanded (i.e., fluidized) by the combined action of upward hydraulic flow, together with an air supply. The carbon adsorbs nonbiodegradable organic materials and provides support for growth of the biological film.

Laboratory-scale tests found that biologically activated carbon was effective in removing biologically degradable organics, organics that are not normally degraded in a biological treatment process under given treatment conditions (the system provides a large surface area for supporting a very long sludge age), and nondegradable organic substances either existing in the influent or generated during the treatment process (Tsezos and Benedek, 1980).

The powdered carbon/activated sludge system is probably not applicable for treatment of groundwater, since the biodegradable organic content in groundwater would be too low to sustain biological growth of activated sludge. A fluidized bed of biologically activated carbon may be suited for treatment of contaminated groundwater since, unlike the activated sludge system, biological growth will be attached to the carbon and no sludge recycle will be necessary. The process is also adaptable for use with other processes, such as wet-air oxidation, which may break complex nonbiodegradable organic compounds into smaller molecules of biodegradable material.

Although biological activated carbon or wet-air oxidation may not be a feasible process by itself, the combination process scheme shown in Figure F-1 may be technically and economically feasible for treatment of dilute contaminated groundwater (Tsezos and Benedek, 1980).

Aerobic biological activated carbon will have performance results similar to those of a typical granulated carbon treatment system. The treatment is enhanced by the coresident biota. Whether the groundwater contaminants will

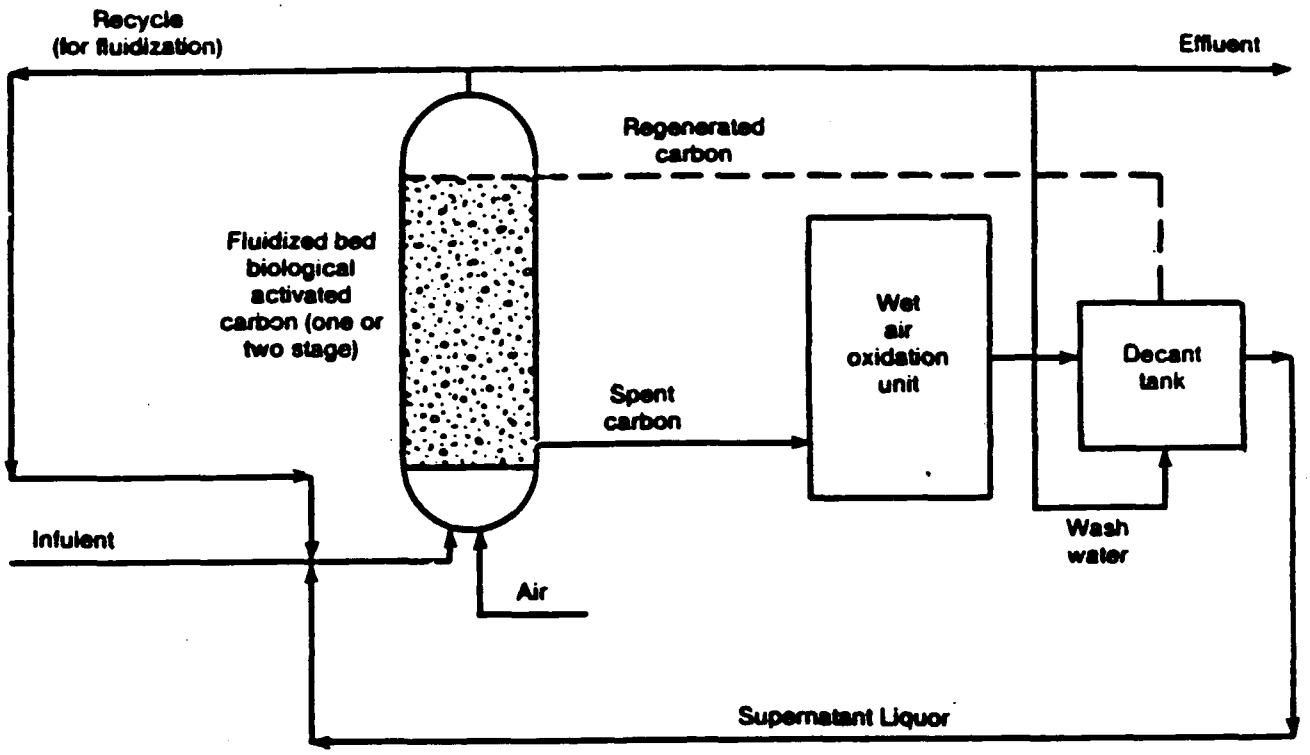



Figure F-1. Biological Activated Carbon/Wet-air Oxidation Combination Process Schematic (Tsezos and Benedek, 1980)

remain at a high enough organic content to sustain the biological growth must be answered on a site-specific basis.

This technology effectively removes contaminants such as volatile organics/solvents, explosive-related organics, and heavy metals.

1.1.2 Anaerobic Activated Carbon Filter Systems (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Khan, Suidan, and Cross, 1981; Suidan, Cross, Fong, and Calvert, 1982)

This filter system removes organics from a contaminated aqueous medium by adsorption or degradation under anaerobic conditions. It combines the advantages of the energy-efficient anaerobic filter process with the long retention of refractory organic compounds by the carbon medium. This medium also provides a surge against shock loading conditions by adsorbing the organic matter out of solution during periods of increased feed concentrations and releasing them back into solution as concentrations decrease.

The anaerobic carbon filter differs from the regular carbon adsorption system in having a significantly longer (20 to 100 times) hydraulic contact time to permit growth of methane-forming microorganisms. Removal of organic contaminants is through adsorption and conversion of organic materials to methane.

A laboratory-scale study, with three-stage anaerobic carbon filters, was conducted to treat coal gasification wastewater containing high concentrations of phenols (Khan, Suidan, and Cross, 1981; Suidan, Cross, Fong, and Calvert, 1982). The system could treat phenol concentrations up to 1,000 mg/l, with 90 to >99 percent phenol removal.

There is a lack of data for applying an anaerobic carbon filter to treat dilute, low-organic strength waste water, such as contaminated groundwater, although the method is considered very promising (Bove, Lambert, Lin, Sullivan, and Marks, 1984).

Figure F-2 shows a schematic diagram of an experimental anaerobic activated carbon filter (Bove, Lambert, Lin, Sullivan, and Marks, 1984). An advantage of the process is a very long service life (two or more years) of the granular activated carbon. However, the carbon might become fouled by biological growth. Safety precautions are necessary in handling methane gas generated from the system. Highly toxic hydrogen sulfide can be generated in both the liquid and gaseous phase. This material may also interfere with the effectiveness of the carbon adsorption process by precipitation and fouling of carbon particles with metal sulfides.

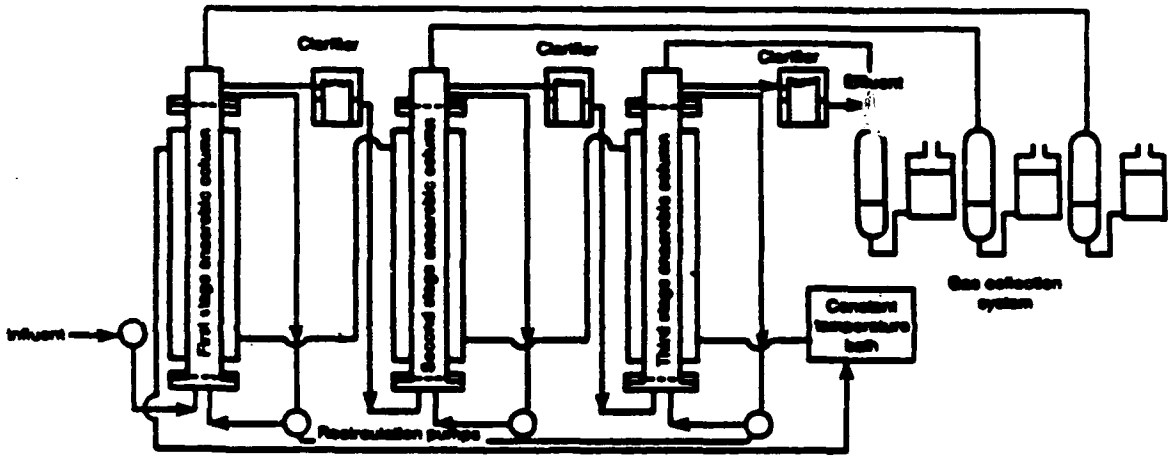



Figure F-2. Experimental Anaerobic Activated Carbon Filter (Bove, Lambert, Lin, Sullivan, and Marks, 1984)

1.2 SOLVENT REFLUXING (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Jhaveri and Mazzacca, 1982)

Solvent refluxing is designed to eliminate hydrocarbons and halogenated hydrocarbon contaminants from groundwater and soil, applying the principles of solvent extraction and enhanced biodegradation.

Contaminated groundwater is pumped to the surface where it is treated using an appropriate removal technology. Nutrients to enhance biodegradation in the soil and groundwater are added to the treated groundwater, which is then reinjected into the contaminated site. This water initiates *in situ* biodegradation and serves as a solvent, dissolving water-soluble contaminants. The groundwater collects in the dewatering wells where it is again pumped to the above-ground treatment system to complete the process cycle.

A system has been developed by Groundwater Decontamination Systems, Inc., (GDS), and has been used to reclaim a site in Linden, NJ, (see Figure F-3) (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Jhaveri and Mazzacca, 1982). The system has decontaminated soils and groundwater of methylene chloride, *n*-butyl alcohol, acetone, and dimethylaniline on a commercial scale. Biological sludge is separated in the settling tank and will require further treatment and disposal. The existing operation treats 12,000 gal/day of groundwater at a cost of $0.05/gal. This includes amortization of capital costs for five years.

This technology effectively removes contaminants such as volatile organics/solvents, explosive-related organics, and heavy metals.

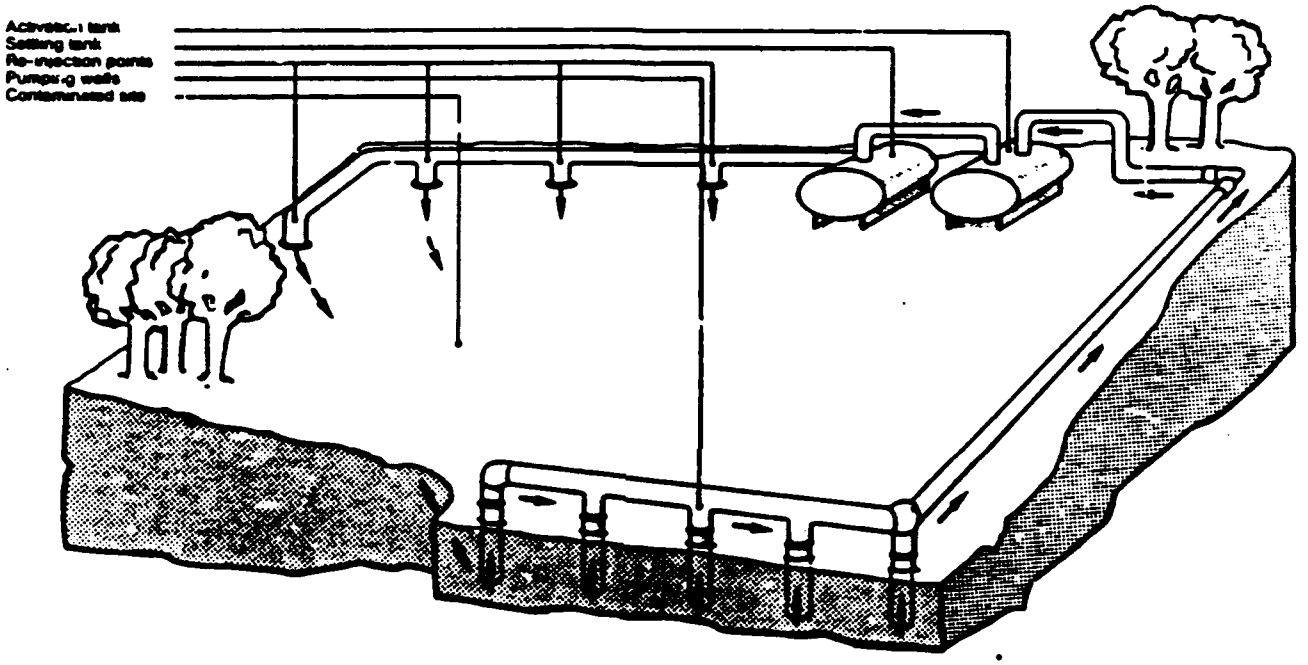



Figure F-3. Process Schematic of the GDS System (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Jhaveri and Mazzacca, 1982)

## 1.3 AEROBIC FLUIDIZED-BED BIOLOGICAL TREATMENT (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Environmental Protection Agency, 1983)

These treatment systems have been developed to remove organics and solvents from contaminated aqueous streams. They combine the features of activated sludge and fixed-film biological processes.

Figure F-4 shows a typical system, which comprises a fluidized-bed reactor and oxygenator; a sand-biomass separator; and feed, recycle, and chemical-addition tanks. Oxygen, required for carbonaceous BOD removal or nitrification, is dissolved in the influent stream prior to entry into the fluidized reactor. The oxygenation system and influent distribution system, both proprietary devices, allow the transfer of at least 50 mg of oxygen/l to the fluid phase. If the oxygen demand of the influent exceeds the level of available oxygen, then effluent recycle is required.

To control the fluidized bed at a specific height, a fraction of the biomass coating is separated from the sand particles by means of a vibrating screen. The sand particles are returned to the bed for reseeding, and the biomass is removed as excess sludge.

The system requires less land area and contact time than other biological treatments because higher volumetric loading rates are possible. Organisms are fixed in the system, as in a trickling filter, and are claimed to be capable of providing greater process stability in handling shock and toxic loads. However, unlike trickling-filtration processes, there is minimal sloughing of biological growth. Therefore, no clarifier or recycle stream of sludge is required.

The key to its effective treatment is the high concentration of active biological organisms provided in the fluidized reactor; i.e., 12,000 to 40,000 mg/l of mixed liquor-volatile suspended solids (MLVSS). This compares with MLVSS values of 1,500 to 3,000 for conventional activated sludge, and 3,000 to 6,000 mg/l for pure oxygen-activated sludge treatment.

These treatments can be used in secondary treatment for BOD removal or in tertiary treatment processes, such as nitrification of ammonia or denitrification of nitrates. They may not work for dilute contaminated groundwater without the addition of supplemental carbon, since the concentration of organic material in the groundwater would be too low to sustain proper biological growth. However, they are probably comparable with most other biological treatment processes in that removal of most of the nonbiodegradable organic material (particularly highly chlorinated compounds) would be quite limited. This process is particularly suited to treatment of relatively high-strength, biodegradable wastewaters. The presence of metals may hinder this process by destroying the active organisms.

Biological process reactors available for water and wastewater treatment can be classified according to the nature of their biological growth (Sutton, 1987). Those in which the active biomass is suspended as free organisms or microbial aggregates can be regarded as suspended growth reactors, whereas those in which growth occurs on or within a solid medium can be termed supported growth or fixed-film reactors.

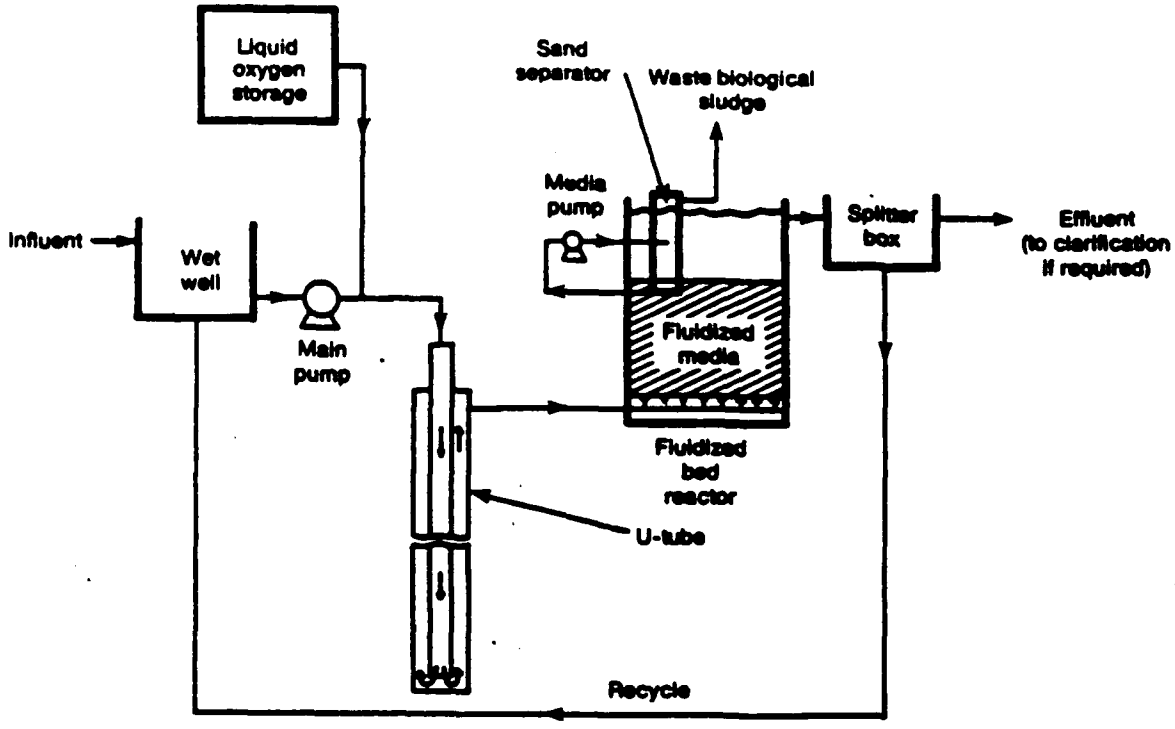



Figure F-4. Process Schematic of an Aerobic Fluidized-bed Biological Treatment System (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Environmental Protection Agency, 1983)

A fluidized-bed reactor represents a highly efficient fixed-film reactor in which biomass build-up occurs on an inert (sand) or active (activated carbon, resin material) fluidized support medium high in external surface area. The principles of the fluidized-bed process have been incorporated into full-scale aerobic and anaerobic configurations through the development, respectively, of the Oxitron and Anitron systems.

In the fluidized-bed process (Figure F-5), the contaminated water or wastewater and recycled effluent pass upward through the bed of medium at a velocity that expands the bed beyond the point at which the frictional drag is equal to the net downward force exerted by gravity. From this point of minimum fluidization, the medium particles are individually and hydraulically supported (Sutton, 1987). They provide a vast surface area for biological growth, in part leading to the development of a biomass concentration approximately five to ten times greater than that normally maintained in a suspended growth system. It may be necessary to control the biofilm thickness to prevent the density of the biofilm-covered medium (bioparticle) from decreasing to the point where bed carry-over occurs. This is accomplished by monitoring the bed expansion optically; carrying out separation of the medium from the biomass, if the maximum specified bed height is reached; and return of the medium to the reactor.

The membrane bioreactor is an advanced suspended-growth reactor in which a high, active microbial concentration (12,000 to 30,000 mg/l volatile suspended solids) is achieved through the use of ultrafiltration for biomass-effluent separation and subsequent recycle to the biological reactors. An example of a commercial setup of the aerobic or anaerobic configurations is represented by the Membrane Aerobic or Anaerobic Reactor System (MARS) (Figure F-6).

These technologies are more favorable than alternative biological systems, in situations where the contamination must be treated as rapidly as possible. Accumulation of a large biomass concentration in the fluidized-bed or membrane bioreactor will allow removal of complex organic compounds efficiently with a short liquid contact time or hydraulic retention time relative to more conventional suspended growth (activated sludge, sequencing-batch reactors, aerobic/anaerobic lagoons) and fixed-film (downflow or upflow packed-bed reactors) systems.

Both technologies allow selective development and retention of microbial populations effective against specific complex compounds. The fluidized bed does it with a biofilm. Biomass loss must be less than the rate of growth of new biofilm, which declines as the concentration of the contaminants is reduced in the reactor, with a loss of efficiency. The MARS bioreactor achieves a large biomass density by absolute and controlled retention of all developed microorganisms.

Tolerance to toxic or inhibitory feed inputs can be further achieved by the use of granular activated carbon (GAC) as the fluidizing medium in the fluidized-bed reactor and the addition of powdered activated carbon (PAC) to the membrane bioreactor. The activated carbon also provides more rapid initial removal upon startup and greater removal of recalcitrant compounds, and it reduces the volatilization of adsorbable compounds.

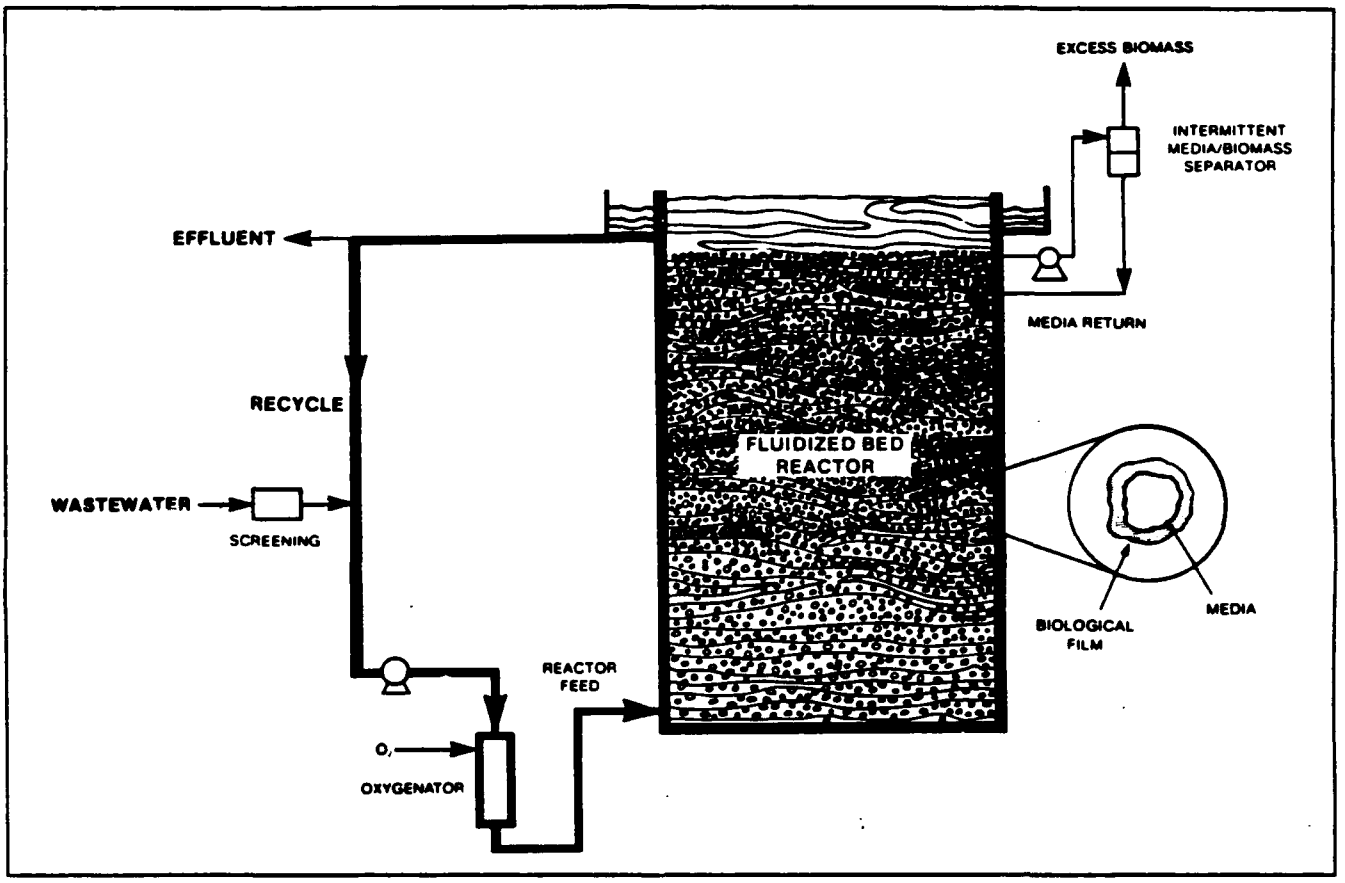



Figure F-5. Fluidized-bed Process (Sutton, 1987)

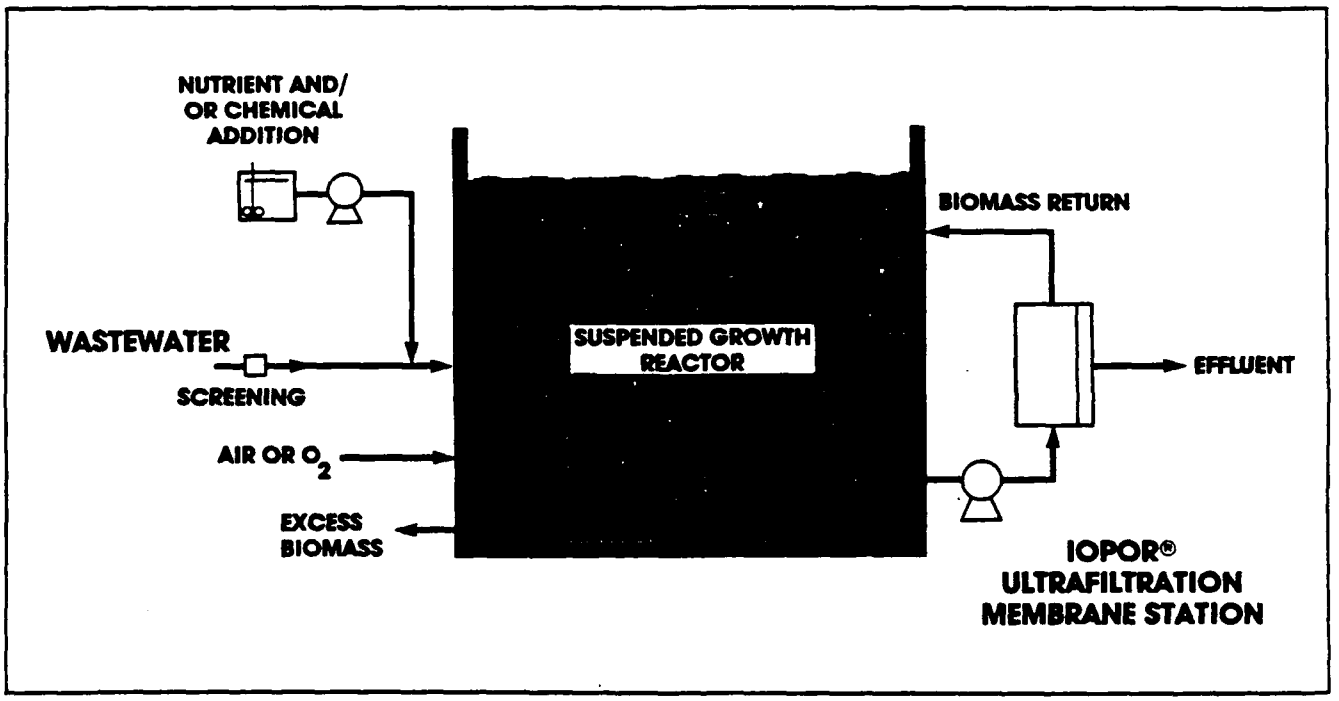



Figure F-6. Membrane Aerobic or Anaerobic Reactor System (MARS) (Sutton, 1987)

Hazardous water and wastewaters containing degradable organic suspended material and emulsified oil and grease are more readily handled in MARS than in the fluidized-bed reactor. MARS is also often more cost-effective for treatment of lower volume water and wastewaters. The biodegradability, adsorbability, and volatility of the waste stream will determine which of the two technologies is more appropriate. Table F-1 compares the factors that govern the selection (Sutton, 1987).

In situations where the treated water is to be disposed of in a municipal treatment plant, the anaerobic versions of the fluidized-bed and the membrane bioreactor systems may be attractive. Anaerobic pretreatment of a leachate source from a landfill site may be more cost-effective than aerobic treatment. Alternatively, series operation of anaerobic and aerobic fluidized- bed or membrane bioreactors may be the most attractive flow scheme.

Oak Ridge National Laboratory has develop a fluidized-bed digester using immobilized aerobic organisms (Roberts, Koff, and Karr, 1988). In lab-scale testing, less than 4 min were needed to reduce phenol levels from 30 mg/l to less than 1 mg/l. This experimental system has handled waste concentrations up to 50 percent.

Table F-1. Factors Governing Selection of Fluidized Bed *versus* Membrane Biological Reactor in the Treatment of Hazardous Water and Wastewater (Sutton, 1987)

| Factor | Fluid Bed Bioreactor (Oxitron/Anitron) *versus* Membrane Bioreactor (MARS) |
|---|---|
| Effluent quality | Membrane bioreactor normally will provide better effluent quality as effluent will contain no suspended solids. |
| Treatment of highly volatile organics | Little or no volatilization/stripping of organics will occur in Oxitron. More will occur in MARS. |
| Treatment of particulate and soluble organics | Particulate organics are handled better in MARS due to retention by ultrafiltration component and subsequent biotreatment. Both reactors handle soluble organics efficiently. |
| Economics | Fluid bed is often more cost-effective than MARS in treatment of high-volume wastewater. |
| Aerobically biodegradable and volatile or semivolatile compounds, such as napthalene, ethylbenzene, toluene, benzene, methyl chloride | Oxitron |

## 1.4 IMMOBILIZED CELLS

There is a promising outlook for the use of immobilized cells for carrying out various types of metabolic transformations on both laboratory and factory scales (Chibata, Tosa, and Sato, 1979). The practicability of industrial processing with immobilized enzymes is already well established and may be enhanced by the use of inorganic supports (Kent, Rosevear, and Thomson, 1978).

Immobilized cells act as a biological catalyst in specific unit process operations (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Polybac Corporation, 1983). Cells that are known to metabolize organics are entrapped in a gel or a polymer matrix, which is usually incorporated into a packed-bed system. Immobilized cells have been found to metabolize carbon significantly faster than free cells. Fermentation products, such as methane, result from the growth of the cells.

This technique has been used in the pharmaceutical industry to produce stereo-specific amino acids, and in the food industry to produce fructose. Immobilized cells have also been studied as a possible means of decolorizing kraft mill effluent (brown color due to lignin). Successful decontamination of groundwater using this technology has not been reported.

There have been preliminary reports of benzene degradation by Pseudomonas putida immobilized in polyacrylamide gel (Somerville, Mason, and Ruffnell, 1977; Mason, Pirt, and Somerville, 1978) and of phenol degradation by Candida tropicalis immobilized in polyacrylamide, polystyrene, and aluminum alginate (Hackel, Klein, Megnet, and Wagner, 1975). The successful use of such systems will depend upon development work to increase productivity, especially when substances of low water-solubility are involved (Drozd, 19809).

The Manville Sales Corporation has developed a means of immobilizing or fixing hydrocarbonoclastic microorganisms to the surface of silicious, diatomite biocatalyst supports (Roberts, Koff, and Karr, 1988). This process can now be used for the treatment and abatement of point sources of dilute aqueous solutions of priority pollutants. For example, phenol in water at concentrations as high as 1500 ppm can be biodegraded to less than 1 ppm at a rate of 1.0 mg/g of R-630 carrier per hour using a mixed population of organisms. R-630 is spherical in shape, has a mesh size of 3/8, and has a mean pore diameter of about 6.6 um.

Figure F-7 shows a schematic flow diagram of a skid-mounted pilot unit developed for this process. The pilot plant consists of mixing and feed tanks in which pH adjustment can be made and to which necessary nutrients can be added. The waste is then pumped to two columns (operating in series or in parallel) containing the selected microorganisms retained in the Celite® diatomaceous earth biocatalyst carrier. The microbial toxic waste degradation occurs in the columns, where air sparging will enhance the reactions. The organisms regenerate themselves, so the process can be operated continuously. The final process would be fully automated with continuous nutrient and pH adjustment of the feed stock and temperature control of the biocatalyst columns.

A pilot study showed the potential for withdrawing and treating contaminated groundwater and, subsequently, discharging it to the sewer system.

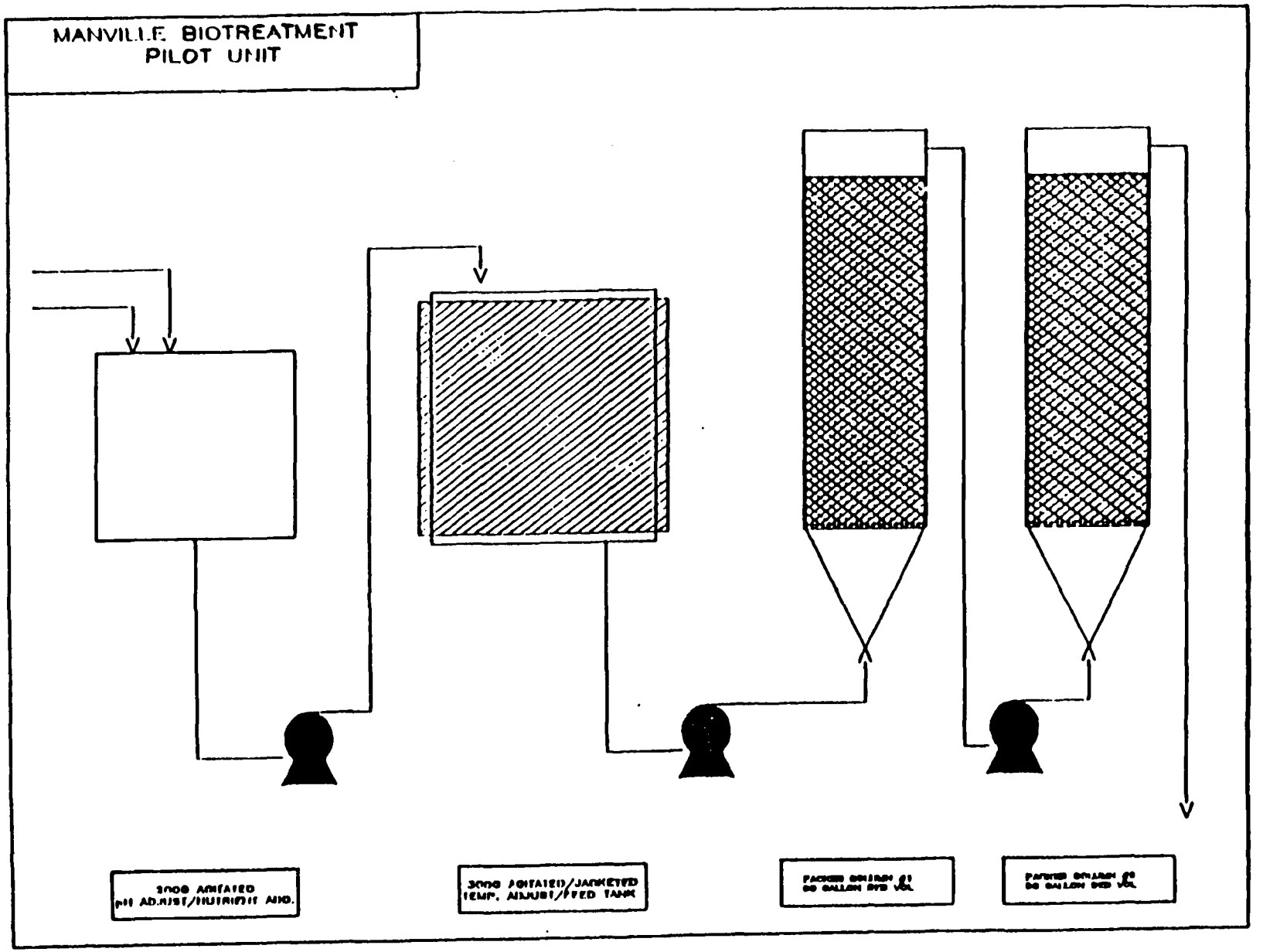



Figure F-7. Manville Biotreatment Pilot Unit (Roberts, Koff, and Karr, 1988)

Another planned modification would use a carrier system that floats to allow the microbes to treat materials spilled on water.

The estimated operating costs range between 1.5 and 2 cents per gallon for the range of capital requirement. More detailed costs associated with this process are given in Appendix C.

## 1.5 IN SITU BIOLOGICAL TREATMENT/LAND TREATMENT

The controlled application of waste materials to soil for degradation by the resident microflora is called landfarming. Landfarming of petroleum wastes has proven to be a successful alternative to incineration when energy conservation and costs are considered. This alternative to in situ biotreatment may be employed in cases where soil permeability is too low for effective groundwater recirculation (Niaki, Pollock, Medlin, Shealy, and Broscious, Draft). The contaminated soil is spread over the surface of the landfarm and incorporated into the top 8 to 12 inches of clean soil. Nutrients can be added at this time, and the soil can be tilled to increase the oxygen level for enhanced biodegradation. Rototilling equipment vigorously mixes the soil, promoting the aeration and mixing process more effectively than disks or bulldozers (Raymond, Hudson, and Jamison, 1976). Tilling the waste material into the soil immediately after application will decrease its chance of migration out of the area. This process has been used extensively as a disposal mechanism for oily sludge.

There are some 197 registered hazardous waste landtreatment facilities in the United States, extending from Alaska to Florida (Brown, 1981). This soil treatment may prove to be the most economical and environmentally sound means of disposing of many complex industrial wastes (Brown, Deuel, and Thomas, 1983). Such disposal can be effective, provided that application rates and scheduling do not result in conditions that allow undesirable components or degradation products to run off or leach through the soil, provided that no materials accumulate to toxic levels in the soil, and provided that volatilization of VOCs is controlled.

Soil disposal of many wastes is effective because the soil has a large surface area on which to adsorb and inactivate waste components (Brown, Deuel, and Thomas, 1983). And, if the soil is properly managed, it also presents an ideal medium for microbial decomposition because of the presence of oxygen, water, and the nutrients needed for degradation of organic constituents. The microbes digest the organic matter and recycle the nutrients into the environment (Brown, 1981). Landtreatment is an effective alternative for wastes that have large concentrations of degradable organic constituents.

Oil sludge biodegradation was found to be optimal at a soil water-holding capacity of 30 to 90 percent, a pH of 7.5 to 7.8, C:N and C:P ratios of 60:1 and 800:1, respectively, and a temperature of 20°C or above (Dibble and Bartha, 1979a). Degradation at 10°C was about one-third that at 40° in another study (Brown, 1981). Here, the optimum moisture content for the highest degradation rate of a refinery waste was found to be 18 percent. At 33 percent moisture (too wet) or 12 percent moisture (too dry), the degradation rate was lower. Addition of micronutrients was not beneficial (Dibble and Bartha, 1979a). Breakdown of the saturated hydrocarbon (alkane and cycloalkane) fraction was highest at low application rates, but higher application rates favored biodegradation of the aromatic and asphaltic fractions. Biodegradation of the latter compounds may be dependent upon a continued presence of saturated hydrocarbons to support the cometabolic biodegradation of the former classes. An application rate of 5 percent (wt/wt) oil sludge hydrocarbon to the soil (100,000 l/hectare) resulted in the best overall biodegradation rate of all hydrocarbon classes, had the highest oxygen uptake rate, and supported the greatest total microbial counts. This was in agreement with results that

determined an optimal application rate of 1.0 g of hydrocarbon per 20 g of soil-sand mixture.

Smaller and more frequent applications yield higher overall biodegradation rates than does infrequent application of large batches (Dibble and Bartha, 1979a; Brown, 1981). They also minimize the adverse effects of toxic oil sludge components and keep the hydrocarbon-degrading microbial population in a continuous state of high activity. At most temperate zone landfarming sites, two 100,000-l/hectare (255 barrels per acre) or four 50,000-l/hectare oil sludge hydrocarbon applications per growing season seem practical.

Application of refinery effluent sludge to soil contaminated with PAHs was followed by bursts of $CO_2$ evolution, and after 25 months, 85 percent of the total PAHs had disappeared (Balkwill and Ghiorse, 1982). Three-ringed PAHs were readily degraded. There was a pattern of increased persistence with increasing molecular weight and condensation.

Landfarms cannot degrade the heavy components of petroleum oils or chlorinated solvents (Niaki, Pollock, Medlin, Shealy, and Broscious, Draft). In an EPA study, 20 to 50 percent of applied oily waste was not biodegradable (Environmental Protection Agency, 1985a). Naphthalenes, alkanes, and aromatics were rapidly degraded, with a half-life of less than 30 days, while refractory compounds accumulated in the soil. The regulatory acceptance of long-term disposal of residual oil and grease in landfarms has not been resolved. In some areas, there is also concern over the air pollution from VOCs released from landfarms. Another EPA study estimates that volatilization accounts for about 6 percent of the total loss of hydrocarbons from a landfarm (*Environmental Protection Agency, 1984a*).

## 1.6 MICROBIAL BIOACCUMULATION OF METALS (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Johnson, Kauffman, and Krupka, 1982; Pierce, 1982b)

Much recent research has been conducted regarding the acclimation of microbes to metallic contamination. Natural or mutant microorganisms are mixed in with a metal-containing aqueous waste and selectively accumulate the metals in their cells. These microbes are subsequently separated from the waste solution as biomass, and the concentrated elemental metals are recovered by burning the microbes.

Microorganisms are known to bioacculumate cadmium, copper, iron, lead, molybdenum, radium, and uranium. Polybac and the O'Kelley Company are studying use of microbes for metals removal from wet-scrubber blowdown streams. B.C. Research has been investigating microbial copper leaching. They have been able to remove 95 percent of the copper from 600-gram batches, with recovery of elemental sulfur. This company plans to develop this process into a 2- to 10-tpd pilot system. However, it is not expected to be commercially usable within the next five to ten years (reported 1983; Short and Parkinson, 1983). McGill University has patents pending on a number of microbial formulations that recover metals from dilute aqueous streams.

With this technology, proper disposal of the concentrated elemental metals in the biomass must be observed.

## 1.7 AQUACULTURE--WATER HYACINTH OR OTHER MACROPHYTES (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Environmental Protection Agency, 1978)

The production of aquatic organisms (both flora and fauna) under controlled conditions has been practiced for centuries, primarily for the generation of food, fibers, and fertilizer. The water hyacinth, Eichhornia crassipes, appears to be the most promising organism for wastewater treatment. However, other organisms are also being studied, among them, duckweed, seaweed, midge larvae, and alligator weeds.

Water hyacinths are large, fast-growing floating aquatic plants with broad, glossy green leaves and light lavender flowers. A native of South America, these plants are found naturally in waterways, bayous, and other backwaters throughout the southern United States. Insects and disease have little effect on the hyacinth, and they thrive in raw, as well as partially treated, wastewater.

Wastewater treatment by water hyacinths is accomplished by passing the wastewater through a hyacinth-covered basin, where the plants remove nutrients, BOD, suspended solids, and metals. Batch treatment and flow-through systems, using single- and multiple-cell units, are possible. Plants harvested from these systems have been investigated as a fertilizer/soil conditioner after composting, as an animal feed, and as a source of methane when anaerobically digested. Detention times in aquaculture basins typically range from 4 to 15 days, depending upon effluent requirements, flow, and climatic conditions.

Aquaculture is most often considered for nutrient removal and additional treatment of secondary effluent. Uptake of metals found at low concentrations (less than 1 mg/l influent) has been reported. Hyacinth treatment may be suitable for seasonal use in treating wastewaters from recreational facilities and those generated from processing agricultural products. Other organisms and methods with wider climatological applicability are being studied.

The ability of hyacinths to remove nitrogen and some phosphorus during active growth periods and to retard algae growth provides potential application in upgrading lagoons, renovation of small lakes and reservoirs, pretreatment of surface waters used for domestic supply, storm-water treatment, demineralization of water, recycling fish culture water, and biomonitoring. Applicability for removal of trace organics or inorganic contaminants from the groundwater remains to be demonstrated.

Climate or climate control is the major limitation in the use of water hyacinths. Active growth begins when the water temperature rises above $10^{o}C$ and is optimum when the water temperature is about $21^{o}C$. Plants die rapidly when the water temperature approaches the freezing point; therefore, greenhouse structures are necessary in northern locations. Water hyacinths are sensitive to high salinity. Removal of phosphorus and potassium is restricted to the active growth period of the plants.

Metals, such as arsenic, chromium, copper, mercury, lead, nickel, and zinc, can accumulate in hyacinths and limit their suitability as a fertilizer or feed material. The hyacinths may also create small pools of stagnant surface water, which can breed mosquitoes; however, mosquito fish in the system will prevent that problem. Spread of the plant must be controlled by barriers

to prevent it clogging waterways. Hyacinth treatment may prove impractical for large treatment plants due to the land requirements. Removal must be at regular intervals to avoid heavy intertwined growth conditions.

No test data are available discussing treatment of contaminated groundwater using aquaculture techniques, although present industrial experience indicates that this technology may be applied successfully for removal of specific groundwater contaminants. This technology effectively removes contaminants such as volatile organics/solvents and heavy metals.

## 1.8 GRASS IRRIGATION (OVERLAND FLOW SYSTEM) (Bove, Lambert, Lin, Sullivan, and Marks, 1984)

Wastewater is applied over the upper reaches of sloped terraces and is treated as it flows across the vegetated surface to run-off collection ditches. The wastewater is renovated by physical, chemical, and biological means as it flows in a thin film down the relatively impermeable slope. A secondary objective of the system is for crop production. Perennial grasses (reed canary, Bermuda, red top, tall fescue, and Italian rye) with long growing seasons, high moisture tolerance, and extensive root formation are best suited to overland flow. Figure F-8 is a diagram of grass irrigation (overland flow system).

Biological oxidation, sedimentation, and grass filtration are the primary removal mechanisms for organics and suspended solids. Nitrogen removal is attributed primarily to nitrification/denitrification and plant uptake. Loading rates and cycles are designed to maintain active microorganisms growth on the soil surface. The operating principles are similar to a conventional trickling filter with intermittent dosing. The rate and length of application is controlled to minimize severe anaerobic conditions that result from overstressing the system. The resting period should be long enough to prevent surface ponding, yet short enough to keep the microorganisms in an active state. Surface methods of distribution include the use of gated pipe or bubbling orifice. Gated surface pipe, which is attached to aluminum hydrants, is aluminum pipe with multiple outlets. Control of flow is accomplished with slide gates or screw adjustable orifices at each outlet. Bubbling orifices are small-diameter outlets from laterals used to introduce flow. Gravel may be necessary to dissipate energy and ensure uniform distribution of water from these surface methods. Slopes must be steep enough to prevent ponding of the run-off, yet mild enough to prevent erosion and provide sufficient detention time for the wastewater on the slopes. Slopes must have a uniform cross slope and be free from gullies to prevent channeling and allow uniform distribution over the surface. The network of slopes and terraces that make up an overland system may be adapted to natural rolling terrain. Use of this type of terrain will minimize land preparation costs. Storage must be provided for nonoperating periods. Run-off is collected in open ditches. When unstable soil conditions are encountered or flow velocities are erosive, gravity pipe collection systems may be required.

Groundwater that has a metallic content that may contaminate either native soils or plants should be pretreated.

A common modification of the distribution method utilizes sprinklers. Recirculation of collected effluent is sometimes provided or required. Secondary treatment prior to overland flow permits reduced land requirements by as much as two-thirds.

Grass irrigation is used extensively in the food processing industry. The process is applicable as secondary or tertiary treatment of municipal wastewater, particularly in warm, dry areas. It is limited by soil type, crop water tolerances, climate, and slope of the land. Steep slopes reduce travel time over the treatment area and, thus, treatment efficiency. Flat land may require extensive earthwork to create gentle slopes. Ideally, the slope should be 2 to 8 percent. High flotation tires are required for equipment. The cost

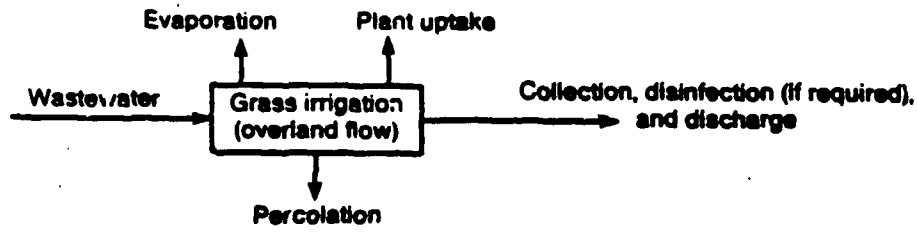



Figure F-8. Diagram of Grass Irrigation (Bove, Lambert, Lin, Sullivan, and Marks, 1984)

and impact of the earthwork required to construct terraced slopes can be major constraints. Application is restricted during rainy periods and stopped when the temperature approaches freezing. Many stages have regulations regarding preapplication treatment, minimum buffer zones, and control of public access The process may not be applicable to the treatment of groundwater containing hazardous organic or inorganic and additional groundwater pollution, unless the site is located immediately above the zone of existing polluted groundwater.

There is a lack of data on removal of toxic organic and inorganic compounds by the grass irrigation method. Grass harvested from irrigation treatment of contaminated groundwater may not be suitable for cattle feed. The process consumes little energy if sufficient head is available, but requires a long-term commitment of a large land area.

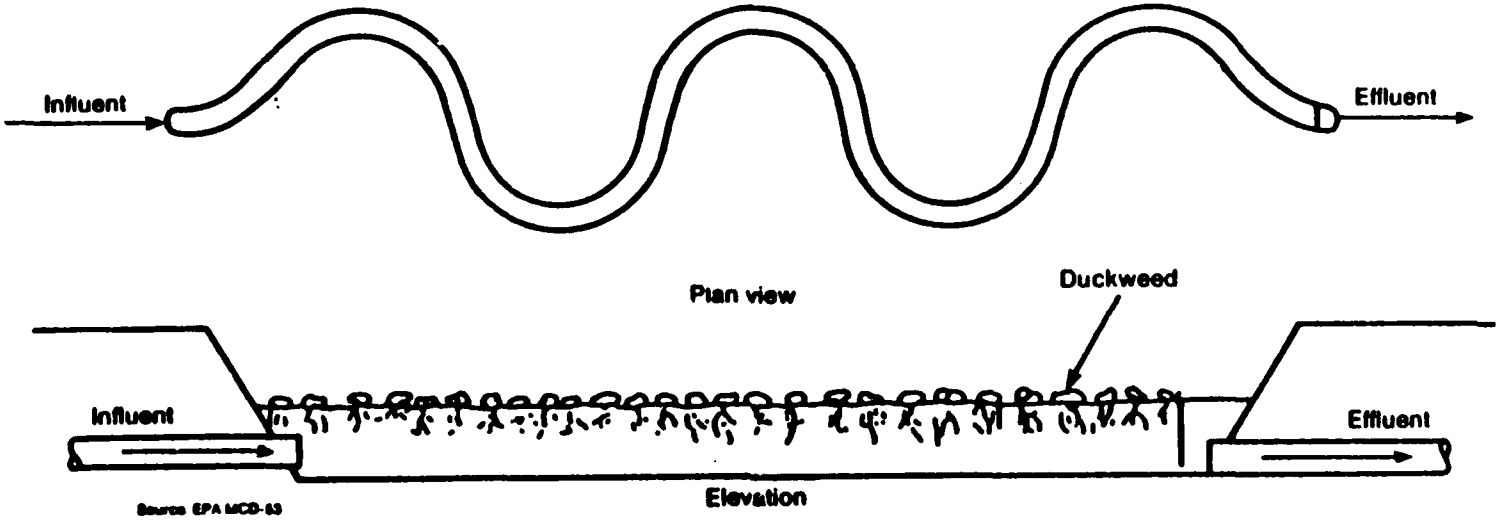
1.9 WETLANDS (Bove, Lambert, Lin, Sullivan, and Marks, 1984; Environmental Protection Agency, 1978)

Natural wetlands, both marine and freshwater, have inadvertently served as natural waste treatment systems for centuries, however, in recent years, marshes, swamps, bogs, and other wetland areas have been successfully utilized as managed natural "nutrient sinks" for polishing partially treated effluents under relatively controlled conditions. Constructed artificial wetlands can be designed to meet specific project conditions while providing new wetland areas that also improve available wildlife wetland habitats and the other numerous benefits of wetland areas. Managed plantings of reeds (e.g., Phragmites species) and rushes (e.g., Scirpus and Schoenomites species), as well as managed natural and constructed marshes, swamps, bogs, and cypress forests, have been demonstrated to reliably provide pH neutralization and some reduction of nutrients, heavy metals, organics, $BOD_5$, COD, suspended solids, fecal coliforms, and pathogenic bacteria (see Figure F-9).

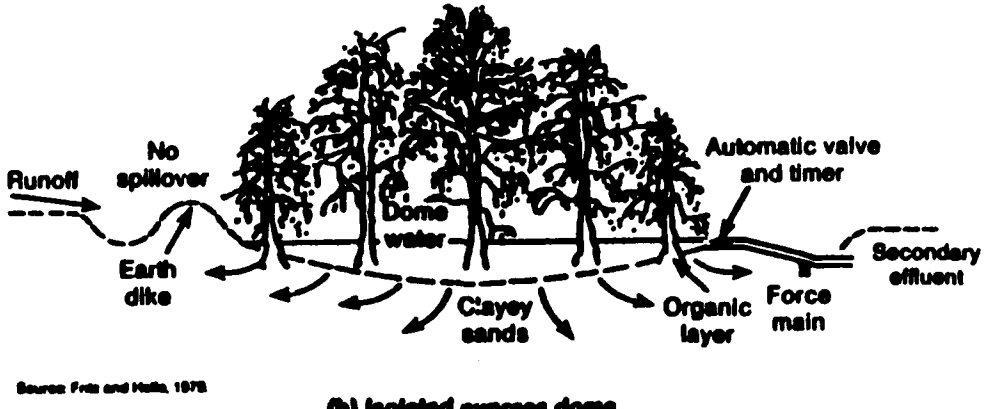
Wastewater treatment by natural and constructed artificial wetland systems is generally accomplished by sprinkling or flood irrigating the wetland area with wastewater or by passing the wastewater through a system of shallow ponds, channels, basins, or other constructed areas where the emergent aquatic vegetation has been planted or naturally occurs and is actively growing. The vegetation produced as a result of the system's operation may or may not be removed and can be utilized for various purposes; e.g., composted for use as a source of fertilizer/soil conditioner, dried or otherwise processed for use as animal feed supplements, digested to produce methane, or eventually harvested as valuable timber.

A wetland is mainly used for polishing treated effluents. Use is highly site-specific and depends upon soil, climate, and wastewater, or contaminated groundwater characteristics. The method is not suited to areas where it is subject to freezing. Use of a wetland for treatment of groundwater contaminated with toxic or hazardous materials may not be environmentally acceptable due to the potential risk of spreading dangerous chemicals to a much larger area for a prolonged period of time. However, a wetland may still be considered for use as a polishing treatment after the majority of the toxic compounds have been removed by other treatment methods.

If a natural site is available, a wetland can offer low-cost treatment while requiring a very low level of energy. However, when it is used for treatment of contaminated groundwater, the system potentially becomes a liability, and is also likely to expose operators to toxic substances.




(a) Constructed wetland planted with duckweed




Source: Frets and Hollis, 1975

(b) Isolated cypress dome.

Figure F-9. Use of Wetlands as Natural Waste Treatment Systems (Bove, Lambert, Lin, Sullivan, and Marks, 1984)

1.10 ANAEROBIC FLUIDIZED-BED BIOLOGICAL TREATMENT (Slonim, Lien, Eckenfelder, and Roth, 1985)

An anaerobic recycle fluidized-bed reactor was used as a pretreatment stage followed by an activated-sludge reactor, as the aerobic treatment stage, for the removal of 4,6-dinitro-o-cresol (DNOC) from wastewater. The DNOC was completely converted during the anaerobic pretreatment stage (anaerobic bed effluent DNOC concentration <1 mg/l, when the influent DNOC concentrations were as high as 600 mg/l). COD removal was approximately 25 percent after this stage and was further reduced by the aerobic stage.

Batch tests employing controlled feed to the anaerobic microorganisms provided data for establishing a range of DNOC loading rates. A pilot plant consisted of an anaerobic upflow sand filter and an activated sludge unit (Figure F-10). Prewashed sand was used as a matrix for the attachment of microorganisms. The temperature of the column was maintained at 36.5°C. The anaerobic upflow sand filter column was operated continuously with a recycle flow rate maintained at about 3,000 l/day. This column was initiated with several inoculations of seed from a sludge digester from a local municipal sewage treatment plant. Operating parameters and performance were carefully monitored. The off-gas was also analyzed and measured.

DNOC could not be used as a sole carbon source by the anaerobic bacteria, and degradation required a readily biodegradable cosubstrate, such as sucrose. There appears to be a relationship between influent sucrose concentration and the performance of the anaerobic pretreatment stage. The ratio of sucrose to DNOC of 2:1 or higher resulted in a 95 to 100 percent conversion of DNOC in this stage. DNOC was not cometabolized when the ratio was less than 2:1.

Other degradation-resistant aromatic compounds, such as recalcitrant phenolic or nitroaromatic priority pollutants, require innovative treatment processes for their removal from wastewater and may also prove to be treatable by this method.

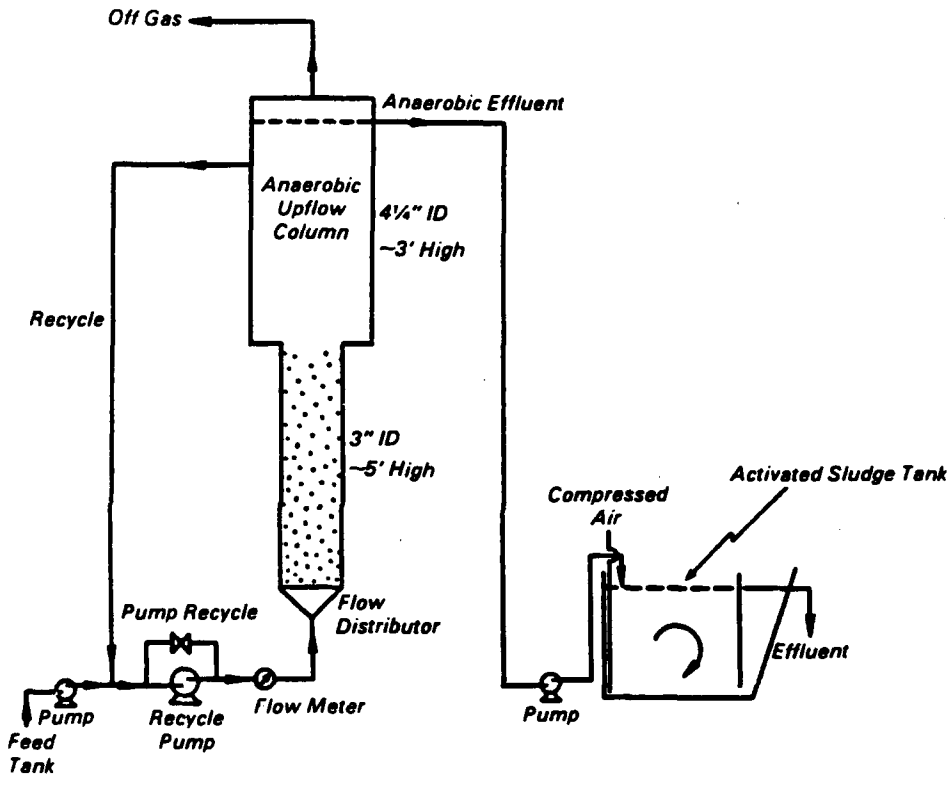



Figure F-10. Schematic of Pilot Plant Apparatus for Anaerobic-aerobic Continuous System (Slonim, Lien, Eckenfelder, and Roth, 1985)

## 1.11 FERMENTOR

If oil to be degraded is collected and placed in a fermentor, biodegradation can be stimulated by adding appropriate microorganisms (Atlas, 1977). The environmental conditions can then be optimized for these organisms, thereby reducing the opportunity for competition between indigenous and seed microorganisms. The main problem involves the impracticality and cost of transporting oil pollutants or oily wastes to the fermentor. The only practical application would be if oily wastes were used that could be easily transported to the fermentor and converted to useful by-products. This cost factor places a further restriction on microorganisms considered as seed. They must degrade petroleum components, as well as convert the oil to useful products. One such useful product would be single-cell protein (Chepigo, Boiko, Gololobov, Kryuchkova, Vorob'era, Rozhkova, Fisher, Pokrovskii, and Krotchenko, 1967). Several hydrocarbon-utilizing yeasts, such as Candida species, have been considered. Not all waste oils are suitable substrates, and nonhydrocarbon substances, such as methanol, are generally considered better for protein production (Rosenberg, Englander, Horowitz, and Gutnick, 1975).

The ballast tanks of oil tankers could be used as fermentors (Atlas, 1977). In this container, seed organisms would not less competition and the environment could be controlled, although this may not prove to be economical. Even with specific seed organisms, the degradation may not be complete, and the ballast water discharge with oily wastes would still have to be disposed of.

## 1.12 SEWAGE TREATMENT

### 1. Activated-sludge Treatment

Such a treatment constitutes its own ecosystem (Atlas, 1977). Oil wastes were often dumped into municipal sewage systems in the past (Itoh, Ohguchi, and Doi, 1968). Many of the organisms in activated sludge are capable of metabolizing hydrocarbons; however, they do not generally extensively degrade petroleum hydrocarbons. It has been shown that hydrocarbonoclastic microorganisms may be added to activated-sludge tanks to degrade the oily wastes. There is an even antagonism between such organisms in activated sludge. Within organic-rich sewage, substrates other than hydrocarbons (e.g., proteins and carbohydrates) may be preferentially attacked, rather than the hydrocarbons (Atlas, 1977). Also, many of the oils found in sewage systems have high concentrations of heavy metals, which may be toxic to microorganisms. It is likely that a hydrocarbonoclastic microorganism would be able to compete and survive in this complex ecosystem. However, rather than seed microorganisms into an activated-sludge treatment facility, it would be better to remove oily wastes separately (Ludzack and Kinkead, 1956; Environmental Protection Agency, 1971) in a specialized secondary tank for oil degradation in a sewage treatment facility that is receiving oily wastes.

### 2. Sewage Lagoons

Some industrial plants with large amounts of oily wastes have set up sewage lagoons for their treatment (McLean, 1971). Proper aeration and additional nutrients will be required for effective petroleum biodegradation in these lagoons. This may require buffering and inclusion of algae in the seed mixture to provide continuous oxygen. Biodegradation of oil in lagoons may even be effective for oil with a large proportion of asphaltenes.

Air can be supplied using forced aeration, which can be used to supply oxygen in lagoons and many freshwater environments (Rosenberg, Englander, Horowitz, and Gutnick, 1975).

Some commercial mixtures of microorganisms have been marketed for use in degrading oil in such lagoons and other situations. Azarowicz and Bioteknika International, Inc., has patented selected bacteria and fungi appropriate for treating oil spills (Azarowicz, 1973).

## 1.13 COMBINATION AEROBIC REACTORS/MICROBIAL ADSORPTION (Kobayashi and Rittmann, 1982)with wastewater

A scheme for separating and treating readily degradable and refractory compounds has been proposed (see Figure F-11). The degradable compounds follow the left path and are treated directly in reactors, while the relatively refractory compounds are removed by sorption. Depending upon the character of the waste, the reactors will be either aerobic or anaerobic, chemotrophic or phototrophic, or a series of several types.

Removal by sorption to microorganisms of compounds not readily biodegraded can extend the detention time in a treatment system for relatively refractory compounds, can concentrate the substance, and can be a means to transfer compounds from one environmental condition to another without making it necessary to deal with the entire volume of wastewater. Once concentrated and removed from the water, refractory compounds can be disposed of by other means, such as incineration or burial. The "relatively" recalcitrant compounds can be effectively degraded if they are first sorbed.

Photosynthetic organisms appear to be potentially valuable when initial sorption is required, because large populations can be readily developed, even under low organic nutrient conditions. A generalized scheme for use of photosynthetic organisms is presented in Figures F-12 and F-13. Cell populations would be initially developed by photosynthetic activity. Sorptive processes would then operate to bioaccumulate the compounds. In addition, heterotrophic activity (with or without the mediation of light), or reductive dehalogenation brought about by the phototrophs, could biotransform the compounds. Once the initial, limiting reactions are performed by the phototrophs, products could be further biodegraded by other organisms.

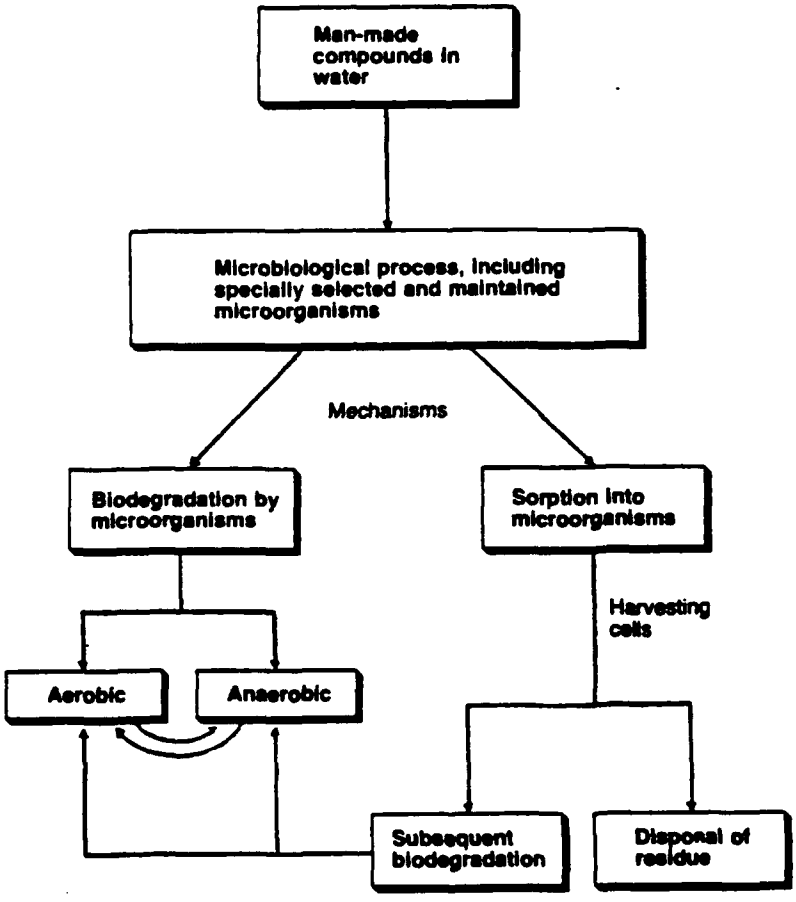



Figure F-11. Biochemical Removal of Man-made Organic Compounds from Water (Kobayashi and Rittmann, 1982)

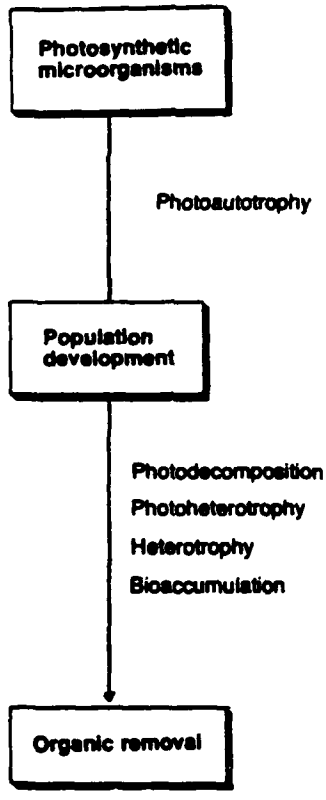



Figure F-12. Removal of Organic Contaminants with Photosynthetic Microorganisms (Kobayashi and Rittmann, 1982)

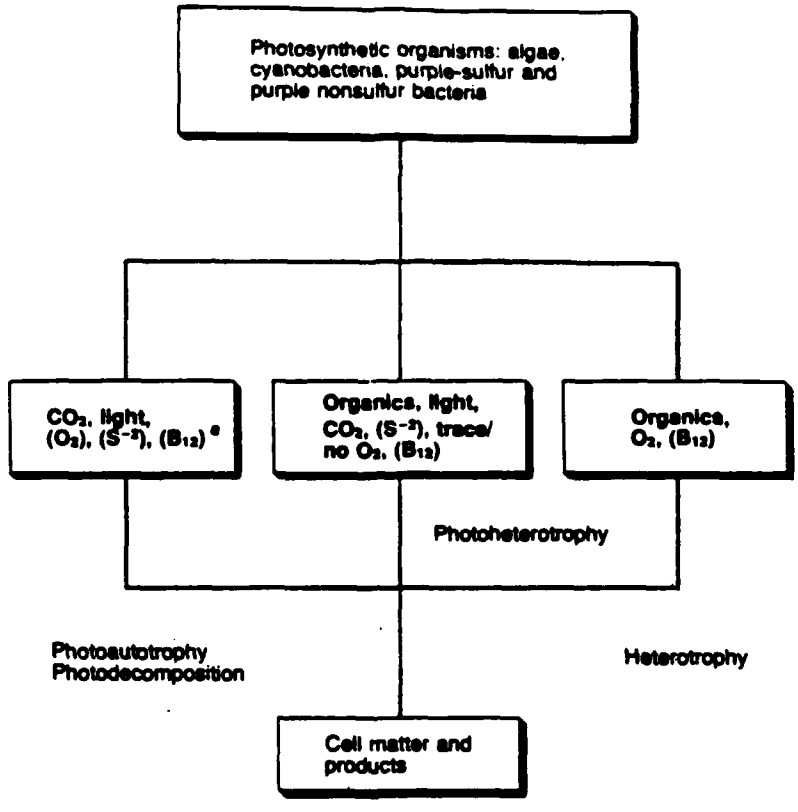



Figure F-13. Mechanisms for Photosynthetic Organism Growth (Kobayashi and Rittmann, 1982)

## SECTION 2

## TEST PLAN FOR DEVELOPING NEW TECHNOLOGIES

A test plan for application in groundwater decontamination will be different for each technology, depending upon its stage of development (Bove, Lambert, Lin, Sullivan, and Marks, 1984). Three testing schemes are briefly outlined here:

1. Screening study (test of concept)

A screening study answers the question, "Does the treatment system using the key unit process work?" The focus is on the key unit process. No attempts are made to derive other than order of magnitude cost estimates. No optimization work is conducted.

2. Bench and breadboard studies (proof of concept)

A bench study is at a larger scale than a screening study. Other unit processes may be linked with the key process. Actual samples may be used in contrast to specifically prepared samples. The question addressed is, "How well does the treatment system work using the key unit process?" A refinement of screening study cost estimates can be made. Information gathered can be applied to either breadboard or pilot systems.

A breadboard study is intermediate between bench- and pilot-scale studies, and may not be required in most cases. This is the first time a complete treatment system is assembled. All unit processes envisioned for full-scale application are present and linked hydraulically and mechanically. There may not be an electrical/control system linkage/integration at this stage. More accurate cost information can be developed. Parametric testing for similarity and scale-up is conducted. A sensitivity analysis of the design and operational variables is completed, and the requirements for an integrated control system are formulated. Real groundwater is used, and the effects of interference are studied.

3. Site demonstration (pilot-scale verification of concept)

Pilot-scale studies use a mixture of commercially available and specifically fabricated equipment of a size that is an economic compromise between the cost of testing and the reliability of scale-up information. A complete treatment system is made operational at some predefined fraction of full-scale. The pilot system is over-instrumented compared with full-scale for the purpose of collecting data.

Hydraulic and mechanical subassemblies may be designed for greater flexibility than expected in a full-scale system for the purpose of allowing investigators to easily reconfigure the system without additional equipment fabrication. Questions addressed at this stage include the following:

1. What are the projected full-scale economics?

2. What is the full-scale optimum design?

3. What are full-scale optimum operational characteristics?

4. What are the human factor implications?

5. What is the configuration of full-scale integrated control?

6. What are the implied impacts on:

   a. Safety

   b. Environment

   c. Establishment/disestablishment

   d. Operator training

   e. Local union constraints

   f. Operation (GOCO, GOGO, COCO)

A test site for research on in situ methods of groundwater decontamination does not exist (as of 1984; Bove, Lambert, Lin, Sullivan, and Marks, 1984). Such a site would involve containment of groundwater flow. Facilities should be available for conducting physical and chemical process testing, either within an aquifer or at an above-ground collection point. Such a site could either be at an actual contaminated aquifer or at a specifically constructed facility. An in situ test site could be established as a joint effort among agencies with parallel interests. It would permit investigations and process development under controlled field conditions and over the period of time required for pilot-scale verification of concept.

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